BREEDING FOR PAPAYA RINGSPOT VIRUS TOLERANCE IN SOLO PAPAYAS, *Carica papaya* L.

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ABSTRACT

A high degree of Papaya Ringspot Virus (PRV) tolerance was observed in a dioecious Carica papaya, line 356, introduced from Florida. Upon infection with the virus, plants of line 356 produced mild leaf, stem and fruit symptoms. Fruit distortion was not observed on these plants. Selected females of line 356 were crossed with 'Kapoho', 'Higgins' and 'Waimanalo' to produce lines 402, 410 and 403, respectively. The level of virus tolerance in these hybrids was observed to be intermediate between the 356 and solo parents. A few selected F2 plants had significantly higher PRV tolerance than the solo parents and only a few trees produced distorted fruits. The F2 plants from 402DF1 and 410AF1 were more tolerant to PRV than 403F1 and derived F2's. Selected 410AF2 trees had the highest virus tolerance among the hybrids but fruit quality was unacceptable. The 402DF2 plants were intermediate in virus tolerance, but produced fruits with good eating quality.

The cool climate at the Olinda station in Maui provided good growing conditions for 5 wild Carica species and one interspecific hybrid, but did not permit successful interspecific hybridization between Carica papaya and other species. Carica papaya was crossed with C. pubescens, C. monoica and a C. cauliflora x C. monoica hybrid, resulting in fruits with a few seeds, but no viable hybrid plant was produced.

The extraction buffer for C. papaya in ELISA was not suitable for PRV detection in other Carica species. Leaf homogenates from C.

pubescens and *C. microcarpa* were observed to have a certain compound that interfered with PRV detection when mixed with a PRV standard. The higher the concentration of *C. pubescens* homogenate in the mixture, the higher the interference effect. This interference compound appeared to interfere with the PRV-γ-globulin, is not protein specific and can be destroyed by heat.

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I. INTRODUCTION

The papaya industry is one of the most important components of diversified agriculture in Hawaii. In 1983 the industry produced 61.4 million pounds of papaya worth $11.6 million (Statistics Of Hawaiian Agriculture, 1983). 'Kapoho solo', grown in the Puna district of Hawaii, is the major papaya cultivar.

On the island of Oahu, due to rapid urbanization of available land, a very limited area has remained for agriculture. Furthermore, growing papaya on this island is difficult due to the presence of a virus disease. This papaya virus was formerly known as Papaya Mosaic Virus (PMV), but it is now known as the Papaya Ringspot Virus (PRV) (Gonsalves & Ishii, 1980). It is the same virus described as PMV in Puerto Rico and India (Adsuar 1946, 1950, 1972; Capoor & Varma, 1948), PRV in Venezuela, and the Distortion Ringspot Virus (DRV) in Florida (Gonsalves & Ishii, 1980).

Papaya plants infected with PRV show a mosaic mottling on leaves, and leaf lobes may be distorted with puckering and blistering. The production and quality of fruit from diseased plants decline as the tree weakens. The surface of the fruit may be severely disfigured and marked with green and brown-colored ringspots. Infected trees eventually die.

There is no cure for this virus disease. Infected trees should be rogued and destroyed to prevent further spread of the virus. The Hawaii Department of Agriculture has successfully eradicated PRV from the island of Maui and contained its spread on Hawaii by an intensive

roguing program. The eradication program was successful in the Puna region because of its isolation from urban areas and because papaya is the only crop grown on the papaya plantations. However, on the island of Oahu, as well as in many foreign countries, diversified and intensive farming is practiced in areas surrounding the city and any organized eradication effort is much more difficult and costly. Papayas and cucurbits grown in backyards of island residences are one of the major reservoirs of the papaya ringspot disease. Home gardeners are usually reluctant to cut down the diseased trees, especially those that are producing fruit. This results in a continuous propagation of the virus disease.

The objective of our studies is to develop virus resistant or tolerant solo papayas that can produce a marketable crop in areas suffering from severe papaya ringspot disease. The purposes of this research are : 1) to screen and select virus resistant or tolerant papaya lines; 2) to observe the nature of the inheritance of tolerance or resistance; and 3) to initiate a breeding program to incorporate the disease tolerance into commercial solo cultivars.

II. LITERATURE REVIEW

VIRUS DISEASES OF PAPAYA IN HAWAII

WAIALUA DISEASE

The first virus disease of papaya in Hawaii was discovered in the Waialua and Lualualei areas of Oahu in 1938 (Parris, 1938). The symptoms were described as a reduction in growth, a downward bending of petioles, chlorosis of leaves, yellow margined, necrotic leaf spots and premature leaf abscission. Necrotic spots were frequently observed on petioles and stems often merging into linear streaks. The disease was experimentally transferred to healthy plants through sap inoculation. Symptoms appeared 6 to 21 days after inoculation. The disease was not named, but was later referred to as the "Waialua" disease. This disease prevailed in the Waialua area from 1938 to 1941 (Ishii & Holtzmann, 1963).

THE ORIGINAL RINGSPOT DISEASE

In March of 1945, a second papaya virus disease was discovered in Kailua, Oahu. It was named papaya ringspot disease. The occurrence and the disease symptoms were described by Lindner et al (1945). Jensen (1949 a,b) demonstrated that the disease was caused by a virus and described the virus-vector relationship, disease symptoms, rate of virus movement through the plant and the host range of the disease.

This virus infected only papayas. It failed to establish in 16 plant species representing 12 families when experimentally transmitted by the aphid, Myzus persicae (Jensen, 1949b).

Infection symptoms included puckering of young leaves and irregular mosaic mottling on expanding leaves. The surface of mature green fruit sometimes developed yellow rings with green centers. These fruit spots distinguish the original papaya ringspot disease from all other virus diseases (Holmes et al, 1948; Jensen, 1949a; Holtzmann and Hines, 1965). Disease symptoms were more pronounced during winter and cool periods.

The incubation period of the original papaya ringspot disease was reported to range from 9 to 39 days after inoculation, with an average of 21 days. Vigorously growing plants produced symptoms more rapidly after inoculation (Jensen, 1949a). The original papaya ringspot virus rarely killed papaya plants; it only weakened them (Holmes et al, 1948; Jensen, 1949a).

SPOTTED WILT VIRUS ON PAPAYA

Trujillo and Gonsalves (1976) observed Tomato Spotted Wilt Virus (TSWV) on Carica papaya. The virus caused severe chlorosis and necrosis on young leaves and water-soaked lesions on petioles and stems of papaya plants. Green fruits were covered with oily spots or concentric rings. In well ripened fruit, ringspots remained green in color. Internal necrosis, bleached flesh and hard lumps in the pulp were also reported. Symptoms of TSWV on papaya appeared within six weeks after inoculation. Topping of infected trees was suggested to be useful in controlling the

disease. According to Trujillo (personal communication, 1983), the symptoms reported by Parris for Waialua disease resemble closely those of TSWV on papaya. Spotted Wilt Virus on papaya was reported only in the Waianae area on Oahu (Cook & Milbrath, 1971).

PAPAYA MOSAIC VIRUS- PAPAYA RINGSPOT

The Papaya Mosaic Virus (PMV) was first discovered in Hawaii in 1959 in the Waimanalo valley on Oahu, and by 1961 the presence of the virus threatened the existence of commercial papaya production. Reports of tree losses due to the virus were as high as 75%. The Papaya Mosaic Virus disease of Hawaii (PMV) is currently known as the Papaya Ringspot Virus (PRV). It is the same virus described as the Distortion Ringspot Virus in Florida (Gonsalves & Ishii, 1980). Papaya Ringspot Virus is transmitted by aphid vectors and can be propagated experimentally by mechanical inoculation. It is not seed transmitted (Conover,1964). No resistance to PRV was reported in papaya (Cook & Zettler, 1970; Conover 1964; 1976a; 1976b).

SYMPTOMS OF PRV

Symptoms of PRV on papaya include an upward curling of leaf margins, with chlorotic mottling and blistering of leaf surfaces. Translucent oil spots and chlorotic lesions may be present along leaf veins, petioles and stems. Older leaves may abscise prematurely until only a small tuft of yellow leaves remains (Holtzmann & Hines, 1965). Slightly raised target-like ringspots with green outer rims and brown

centers are found on the fruit surface. These ringspots may range from 4 to 8 mm in diameter (Holtzmann & Ishii, 1963). Ringspots on fruits can be observed as early as two weeks after fruit set (Holtzmann & Hines, 1965). The fruit surface may also be distorted. Fruit distortion usually occurs when papaya plants are infected by the virus during early fruit development. Poor flavor, bitterness and low sugar content are usually associated with infected fruits (Ishii & Holtzmann, 1963; Khurana, 1970).

Symptoms of PRV infection appear within three to four weeks after inoculation. The rate of disease development is influenced by tree age and temperature. Young plants are more susceptible than mature plants (Holling & Brunt, 1981; Capoor & Varma, 1948). High temperatures usually suppress disease symptoms, and low temperatures enhance them (Conover 1962; 1964; Ishii et al, 1961; Ishii and Holtzmann, 1963; deBokx, 1965).

PROPERTIES OF POTYVIRUS

The papaya ringspot virus is classified as a potyvirus based on the particle length and its ability to induce cytoplasmic inclusions in infected plants (Gonsalves & Ishii, 1980; Harrison et al, 1971). A potyvirus particle contains approximately 5% RNA and 95% protein and has a molecular weight range from 3.0 to 3.5 x 10^6. Potyviruses are fairly stable *in vitro* and occur at moderately high concentrations in infected plants. They are probably replicated in the cytoplasm of infected plants. A high degree of host specificity is a characteristic of this

group of viruses. General symptoms caused by potyviruses on dicotyledonous plants include vein clearing, mosaic mottling, puckering, rugosity and sometimes distortion of the leaves. Infected plants may be severely stunted. Flowers on infected plants, especially those with anthocyanin, may show color break on the petals. Fruits may be mottled or deformed. A severe reduction in yield may also result from the virus infection. Examples of potyvirus diseases include Papaya Ringspot, Pepper Veinal Mottle and Watermelon Mosaic (Hollings & Brunt, 1981).

Temperature has a strong influence on symptom expression of potyvirus infections. More serious symptoms develop on plants kept at 10°C to 15°C than those at 25°C. High temperatures generally reduce the severity of symptoms (Stevenson & Rand, 1970).

A decrease in photosynthetic rate, chlorophyll content and number of chloroplasts in infected plants has been reported. A 40% increase in respiration was reported in tobacco leaves infected with Tobacco Etch Virus (Owens, 1957).

PROPERTIES OF THE PAPAYA RINGSPOT VIRUS

PHYSICAL PROPERTIES

Papaya Ringspot Virus is a filamentous, thread-shaped virus with a particle length of 780 to 800 nm, 12 nm diameter and center channel 3 nm wide (deBokx, 1965; Gonsalves & Ishii, 1980; Herold & Weibel, 1962; Zettler et al, 1968). The thermal inactivation point of the virus is 53°C to 56°C; dilution end-point is 10^{-3} to 10^{-4}. Storage life *in vitro* at 24°C is between 8 to 24 hours and the virus may remain virulent

up to 3 days (Capoor and Varma, 1948; Conover, 1962, 1964; Harrison et al, 1971; Ishii & Holtzmann, 1963; Zettler et al, 1968; Chen & Wey, 1979).

HOST RANGE OF PRV

Various studies of the host range of PRV indicate that certain members of the families Caricaceae, Chenopodiaceae and Cucurbitaceae are susceptible to the virus (Namba & Kawanishi, 1963; Conover, 1964; Cook & Milbrath, 1971; Cook & Zettler, 1970; Chen & Wey, 1979; Su & Lin, 1979; Hollings & Brunt, 1981). Virus particles are readily recovered from plants such as cucumber, Cucumis sativa L., muskmelon, Cucumis melo L., watermelon, Citrullus vulgaris Thunb., summer squash and pumpkin , Cucurbita pepo L., and Cyclanthera pedata. Watermelon is the best host for the virus. Aphids that had fed on infected watermelon plants gave the highest successful transmission rate (20%), followed by papaya (8.3%) and cucumber (5.8%) (Namba & Kawanishi, 1966). Chenopodium amaranticolor and Chenopodium quinoa are local lesion hosts (Cook & Milbrath 1971).

In the family Caricaceae, C. papaya, C. cauliflora, C. goudotiana, C. monoica (Conover, 1962, 1964), C. pubescens, C. quercifolia , C. microcarpa and C. parviflora were reported to be susceptible to PRV (Cook & Zettler, 1970; Cook & Milbrath, 1971). Carica cauliflora, C. pubescens , C. X heilbornii n.m. pentagona, C. X heilbornii n.m. chrysopetala, C. X heilbornii n.m. fructifragrans, C. candicans and C. stipulata were reported to be resistant in other studies (Horovitz &

Jimenez, 1967; Malaguti, Jimenez and Horovitz, 1958). Jacaratia spinosa, J. corumbensis (Horovitz & Jimenez, 1967; Cook & Milbrath, 1971; de Zerpa, 1976) and J. mexicana (Cook & Zettler, 1970) were believed to be immune to the virus.

Mekako (1975) reported that in a field of Carica species located adjacent to a field of PRV infected papaya, C. cauliflora did not show visible symptoms of virus infection. Other species such as C. papaya, C. monoica, C. goudotiana, C. parviflora and C. quercifolia were susceptible. None of the Carica species except C. papaya were killed by the virus.

Certain antiviral properties may be present in some of the resistant species (de Zerpa, 1967). When a PRV virus extract was mixed with the leaf extract from Carica X heilbornii, nm. chrysopetala, C. X heilbornii nm. petagona or C. cauliflora, the virulence of the virus was reduced. Only slight viral inhibition was observed in similar experiments with sap from C. papaya and C. monoica.

SEROLOGICAL PROPERTIES

There are possibly two strains of Papaya Ringspot Virus in Hawaii: PRV HA and PRV HB (Gonsalves & Ishii, 1980). Both strains were isolated from papaya plants. Papaya Ringspot Virus HA produces a severe distortion and intense mosaic mottling on zucchini leaves. Papaya Ringspot Virus HB produces a lesser distortion and milder mosaic mottling. Both PRV HA and PRV HB induce severe infections on papaya plants (Gonsalves & Ishii, 1980). Serological tests between PRV HA, the

Florida strain of PRV and Watermelon Mosaic Virus-1 indicates that they are serologically related. Papaya Ringspot Virus HA was observed to react positively with antiserum prepared from WMV-1 in a SDS immunodiffusion assay. Papaya Ringspot Virus HA and WMV-1 produced similar symptoms on squash, C. pepo, but only PRV HA caused local lesions on Chenopodium quinoa , Chenopodium amaranticolor and infected Carica papaya. The serology of the virus and detailed procedures for purification of PRV HA by cesium sulphate density gradient centrifugation has been reported by Gonsalves & Ishii (1980).

Transmission of PRV by aphid vectors was reported to be more effective than sap inoculation. Three out of twenty WMV-1 cross-protected C. metuliferus were infected by PRV-HA when the plants were subjected to feeding by viruliferous aphids (Gonsalves and Namba, personal communication, 1982). No cross protection was observed between the original "Papaya Ringspot" virus and PRV (Ishii & Holtzmann, 1963).

VECTORS AND TRANSMISSION OF PAPAYA RINGSPOT VIRUS

Papaya Ringspot Virus is a non-persistent stylet borne virus transmitted by aphids (Watson, 1946; Kennedy et al, 1962; Brandley, 1964). Myzus persicae Sulz. was identified as the major aphid pest in Hawaii and the major virus vector of PRV and potyviruses (Cook & Milbrath, 1971; Hollings et al, 1981). Other aphid vectors for PRV include Aphis gossypii Glover, A. malvae Koch (Capoor & Varma 1948), A. craccivora Koch, Macrosiphum euphorbiae Thomas and Rhopalosiphum maidis

Fitch (Higa & Namba, 1971). Aphis middletonii Thomas and Amphorophora sonchi Oestlund are minor or occasional pests on papaya.

The virus is most effectively acquired by aphid vectors through superficial stylet insertion into the epidermal layer of the infected plant. Deep probing by the aphid actually reduces the amount of virus acquired (Bradley, 1956). The time required for M. persicae to acquire the virus was reported to range from 10 seconds to 5 minutes. Transmission can occur after 10 seconds of feeding (Cook & Milbrath, 1971; Holtzmann & Hine, 1965; Namba & Kawanishi, 1966). A PRV-carrying aphid can remain viruliferous for 30 to 60 minutes. No transmission was observed 60 minutes after feeding on infected plants (Namba & Higa, 1975; 1977; Namba & Kawanishi, 1966). No latent period between acquisition and transmission was reported. A starving period of one to three hours before acquisition feeding can enhance the success of transmission (Watson 1938). Probing of other vegetation by viruliferous aphids before feeding on papaya can reduce the success of transmission (Namba & Higa, 1975).

Papaya Ringspot Virus and the other potyviruses do not multiply in the aphid. Only the terminal few nanometers of the aphid stylet are involved in the transmission process. Taylor and Robertson (1974) discovered virus-like particles present at the distal 20 nm of the maxillary food canal of M. persicae after a brief feeding on a Tobacco Etch Virus infected tobacco plant. It was also reported that no transmission of potato virus Y occurred if the stylet tip of

viruliferous aphids was irradiated by ultraviolet light or treated with formaldehyde immediately after acquisition feeding.

It was hypothesized that a certain helper component is required for successful aphid transmission of potyvirus. This transmission helper is present in diseased plants and is needed to attach the virus particles onto the appropriate mouth part of the aphids (Govier et al, 1977). This helper substance was reported to be a protein with molecular weight of 100,000 to 200,000. It was precipitated in poly-ethylene glycol (mol. wt. 6,000), but did not form sediment in 100,000 G centrifugation for 90 minutes (Govier & Kassain, 1974a). Activity of the helper component is destroyed when heated at 55°C for 5 minutes or incubated with pronase or trypsin. The specific mechanism of the helper component in virus transmission is not understood (Govier et al, 1977). Experimental results showed that M. persicae failed to acquire purified potato Y virus when fed through a membrane (Pirone & Megahed 1966), but if the purified virus was mixed with a virus free extract from an infected tobacco plant, transmission did occur (Govier & Kassanis 1974a). Piron (1979) suggested that other factors besides the helper component may be involved in aphid transmission of virus.

VIRUS SPREAD IN FIELD

The rate of spread of PRV in papaya plantations depends mainly on the proximity of the source of inoculum, the abundance of alate (winged) aphids and their activity (Hollings & Brunt, 1981; Kuo et al, 1979). In the tropics, the lack of a cold winter and continuous cropping of

susceptible plants increases the chance of early infection by the virus. Papaya Ringspot Virus infected papayas in abandoned fields are the primary reservoir of the disease (Ishii, 1972; Kuo et al, 1979).

Aphid activity is closely related to climatic conditions. Temperature is the most important factor. The optimal temperature for aphids to reproduce is about 26°C. High temperatures generally increase aphid activity and shorten their life cycle. Alate aphids do not normally fly at temperatures below 20°C (Broadbent, 1949). At temperatures around 30°C, alate aphids increase their reproductive activity. Also migration of the aphids occurs with the wilting of the host plants (Broadbent & Martini, 1959). When the mean daily temperature reaches 32°C, aphid activity is greatly reduced. In central South Africa and hot areas in Australia and California, aphids cease to infest potato plants because of the high temperature (Van der Plank, 1944; Grogan et al, 1952).

It is unusual to find aphids colonizing Carica papaya or feeding on the foliage. Probing by the migrating alate aphids is considered the primary cause of PRV spread (Conover, 1964). The highest incidence of virus dissemination in Hawaii occurs during late winter and early spring, when the vector population is most abundant. The rate of aphid movement is also higher after excessive rain or wind (Ishii et al, 1972).

The spread of PRV in a papaya farm usually occurs with the introduction of the virus by aphid vectors from an outside source. It is followed by a secondary spread with the same vectors within the

planting. Intensification of the disease in a field is usually the result of the secondary spread of the disease (Kuo et al, 1979).

Young papaya plants up to one year old are most susceptible to natural PRV infection. Mature plants are less accessible to the visiting aphids because of tree height. In a young papaya planting, rate of spread of PRV was reported to be at a logarithmic proportion of 0.054 trees per day. The secondary spread of PRV from an initial 18 trees (0.7% of the population) to 2,200 trees (88.0%) of the population occurred in 84 days (Ishii, 1972). Long distance spreading of the virus by aphid vectors is very unlikely; a maximum distance of 375 feet was reported by Wolfenbarger (1966). However, this estimate was thought to be too conservative (Ishii, 1972). A one mile wide disease-free belt has been established around the papaya plantations in Puna. This crop-free zone was believed to be effective in preventing the natural introduction of PRV into the area. In general, the width of a crop free belt is determined by wind, floristic and geographic conditions of the region (Namba & Higa, 1977).

PREVENTION AND CONTROL OF THE PRV DISEASE

Papaya Ringspot Virus is a problem of outdoor cultivation. An unscreened glass house can provide a good degree of protection against the introduction of the virus (Hollings et al, 1981). Insecticide spray is not effective in controlling the non-persistent type of virus diseases (Hoyman, 1958; Bart et al, 1960). Roguing the diseased plants as the symptoms become visible can significantly reduce the spread of

the virus (Kuo et al, 1979; Ishii, 1972). Papaya Ringspot Virus was eradicated and kept out of the Puna and Pahala areas of Hawaii and the island of Maui by a vigorous roguing program and strict control of plant movement between islands (Nakasone, 1979). A roguing procedure was recommended by Holtzmann and Hine (1965) in the following manner: 1) spray all infected trees with an appropriate insecticide so that aphid carriers are destroyed; 2) cut and remove from the growing area cucurbitaceous plants and all infected trees, so that the disease cannot spread and all infected plant parts will dry out and die; 3) avoid nearby cultivation of all cucurbitaceous plants, as the virus is found naturally in several species of this plant family; and 4) control aphids with pesticides since they are carriers.

Other measures that are being investigated for the control of PRV disease included cross protection, interspecific hybridization of Carica papaya and PRV resistant Carica species, and the breeding for virus tolerance in Carica papaya.

CROSS PROTECTION

Cross protection occurs when a plant is systemically infected with one strain of a virus and then is protected against infection by other strains of the same virus. This method successfully controlled the Tomato Mosaic Disease of green-house tomato crops in the Netherlands and the United Kingdom (Fletcher & Rowe, 1975), and the Citrus Tristeza Virus on sweet orange in Brazil (Costa & Muller, 1980).

Su and Lin (1979) in Taiwan isolated 2 strains of Papaya Ringspot Virus from papayas by local lesions on C. amaranticolor. The virus strain that produced mild symptoms on papaya was named "Mild Mottle" (M) and the more severe strain was named "Severe Mottle" (SM). Preliminary studies suggested that a possible cross-protection existed between these two virus strains.

No mild strain of PRV occurs naturally in Hawaii (Ishii, personal communication, 1982). Attenuated strains of PRV-HA were created by subjecting the crude sap from PRV-infected squash with nitrous acid (pH 6.0), and inoculating onto Chenopodium quinoa. Two possible mutants, PRV HA 5-1 and PRV HA 6-1, each isolated from a single-lesion on Chenopodium quinoa were reported to produce no symptoms in papaya seedlings under greenhouse conditions. Papaya Ringspot Virus HA 5-1 was observed to protect papaya seedlings against different challenge inoculations with severe strains of PRV (Yeh & Gonsalves, 1984). However, superinfection was reported in large portions of the cross-protected papaya seedlings when young non-expanded leaves or the entire plants were inoculated with PRV HA. The expression of severe symptoms in these superinfected plants occurred 1 to 2 months after the challenge inoculation. To minimize the occurrence of superinfection in the field, it was recommended that papaya seedlings should be inoculated with the protectant strain at the one true leaf stage, and be kept in the green house for about 1 month before being transplanted to the field (Yeh & Gonsalves, 1984).

INTERSPECIFIC HYBRIDIZATION OF C. PAPAYA AND OTHER CARICA SPECIES

Interspecific hybridization between C. papaya and other species is difficult and has not produced very promising results. Mekako and Nakasone (1975) reported unsuccessful attempts to cross C. papaya with C. cauliflora and C. goudotiana. Sawant (1957) studied crossing relations between C. papaya, C. monoica, C. goudotiana and C. cauliflora; he was not able to produce interspecific hybrids involving C. papaya. Horovitz and Jimenez (1967) were unable to incorporate the gene for PRV resistance into C. papaya by crossing C. papaya with C. candicans and C. stipulata. Wolfe and Lynch (1940) had no success in crosses made between C. papaya and Jacaratia spinosa.

Jimenez and Horovitz (1958) classified 6 Carica species into 3 groups according to their ease of hybridization. Carica monoica, C. cauliflora, C. microcarpa and C. pubescens were placed in group 1 of the classification. These species are cross compatible and always produce viable seeds. Carica papaya was placed in group 2 and C. goudotiana was placed in group 3. Crosses made between members in group 1 and C. papaya did not produce any mature seeds, but the immature embryos were believed to be viable if cultured In Vitro. Carica papaya (group 2) and C. goudotiana (group 3) were incompatible.

This classification of crossability in Carica species was later modified into the following categories (Horovitz & Jimenez, 1967). Crosses that:

1) produce no fruit: e.g. C. parviflora x Carica spp.

2) produce fruit that

a) have no seed: e.g. Jacaratia spinosa x Carica spp.

b) have developed seeds:

i) with faulty embryo: e.g. C. papaya x Carica spp.

ii) with faulty endosperm: e.g. Carica spp. x C. goudotiana

c) have no endosperm: e.g. C. monoica x C. stipulata

d) have complete seeds: e.g. C. monoica x C. pubescens

and reciprocal cross.

Mekako (1972) observed that in some of these hard-to-cross species, pollinations were more productive if they were made during the cool season. High temperature and water stress were suspected to reduce the receptivity of female flowers and increase flower drop during the summer season.

Higgins and Holt (1914) claimed success in producing interspecific hybrids of C. cauliflora x C. papaya and C. papaya x C. goudotiana. Warmke et al (1954) reported successful crosses between C. papaya and C. pubescens. Horovitz and Jimenez (1967) produced hybrids of C. papaya x C. cauliflora and C. papaya x C. stipulata, however, these hybrids were lost after field planting. They also obtained three long peduncled females from hybrids of C. papaya x C. pubescens, but these plants failed to produce any back-crosses with C. papaya or C. pubescens. All these F1 progenies produced by crossing resistant and susceptible species were resistant to the Papaya Ringspot Virus. The F2 progenies from crosses between C. monoica (susceptible) x C. pubescens and C. cauliflora x C. monoica were observed to produce a ratio of 3 resistant plants to 1 susceptible plant (Horovitz & Jimenez, 1967).

Khuspe et al (1980) reported a 3:1 ratio of PRV resistance to susceptibility in three thousand F2 plants derived from C. papaya x C. cauliflora hybrids.

VIRUS TOLERANT PAPAYA

All Carica papaya are susceptible to PRV (Cook & Zettler, 1970; Conover, 1976a, 1976b). However, different papaya selections from different areas were observed to have significant variation in their response to the virus. Infection symptoms ranged from severe to very mild in different plants. A breeding program for PRV tolerance was initiated in 1975 by Dr. Robert A. Conover at the Agricultural and Education Center in Homestead, Florida (Conover, 1976). From 95 papaya accessions from the Tropics, 2 dioecious papaya selections were identified as highly tolerant to PRV. One of these lines was introduced by S.E. Malo from Colombia and the other was selected by Harold E. Kendall (Conover & Litz, 1978).

The tolerance of these papayas to PRV may be quantitatively inherited. In the papaya line introduced from Colombia, 4% of the plants showed tolerance to PRV, 11% were mildly susceptible, 80% had severe symptoms and 5% were very severely infected (Conover & Litz, 1981). When the tolerant plants were sibbed and selected for 3 generations, the amount of tolerance in the population increased to 55%, with 37% of the plants showing mild symptoms, 8% severe and none with very severe symptoms (Conover & Litz, 1981). A certain degree of complementary effects were observed between different selected trees,

the ability of a given tree in transmitting PRV tolerance to the progeny was related to the tree it was mated with; parental trees which were rated equally in virus tolerance did not transmit the tolerance equally to their progeny (Conover & Litz, 1978). Since these PRV tolerant plants were dioecious, individual plants selected for high PRV tolerance could not be selfed, both male and female trees had to be selected and sibbed in each generation. The fruit character and quality contributed by the male trees could be evaluated only through the progeny (Conover & Litz, 1978).

III. MATERIALS AND METHODS

Breeding and evaluation of Papaya Ringspot Virus tolerance in Carica papaya has been conducted at the Waimanalo Experimental Farm since the late 1970's, but the investigations reported here were conducted between June 1980 and August of 1984.

The Waimanalo farm is located on the windward side of the island of Oahu at approximately 30 meters (100 feet) elevation. It is exposed to strong north east trade winds. Annual rainfall averages about 76 cm (40 inches), most of which occurs between the months of December and March (Warner, 1972). The soil type in the station is composed of silty clay, a mixture of hydrated halloysite and montorillomite, with high cation exchange and buffering capacity (Awada & Suehisa, 1970).

Twenty selected Carica lines were obtained from the Department of Horticulture papaya breeding project in June, 1980. These selections included PRV resistant species introduced from South America, virus tolerant selections from Florida, India, Hawaii and Okinawa (Table 1). Three planting cycles were observed from 1980 to 1984. The first 2 plantings were arranged in randomized complete blocks. A total of 4 replications with 6 plants per replication was planted for each line. Evaluation and selection of virus tolerant plants was conducted for the parental materials, the F1 and F2 progenies.

Table 1-- List of the original 20 papaya lines evaluated for PRV-tolerance

Line	Description	Sex Type
336	Florida PRV Tolerant Selection	Dioecious
337	" " " "	"
354	" " " "	"
355	" " " "	"
356	" " " "	"
357	" " " "	"
Higgins	Virus Tolerant solo papaya	Hermaphrodite
Kapoho Solo	Susceptible to PRV	"
319F3	45F6 x Higgins	"
318AF3	77-23 x Higgins	"
40F6	Susceptible to PRV	"
342	Okinawa #2	Dioecious
343	Okinawa #4	"
345	*C. cauliflora* (Venez.)	"
347	*C. monoica*	Monoecious
348	*C. pubescens*	Dioecious
349	*C. stipulata*	"
364	*C. cauliflora*	"
365	*C. stipulata*	"
366	(*C. papaya* 'Washington' x *C. cauliflora*) F2 From India	"

All papaya seedlings were grown and maintained in the University of Hawaii Horticulture Facility located in Manoa valley, Oahu. Seeds were sown in #2 horticultural vermiculite. Seedlings were transplanted at full cotyledon stage into 7.6 cm (3 in.) "Jiffy" peat pots. A 4:1 mixture of vermiculite to perlite was used as the growing medium. One tablespoon of dolomite was added into each 113 liters (4 ft^3) of potting mix to supplement the calcium content. A 100 ppm solution of a fungicide containing Metalaxyl was used to drench the medium to prevent seedling damping-off.

Newly transplanted seedlings were kept in a 30% shade screen house for two weeks before be moved into full sun. Seedlings were watered daily. A spray application of a soluble 20-20-20 fertilizer was applied at 15 ml/3.78 liters/week. One teaspoon of 14-14-14 + minor slow release fertilizer was given to each seedling at two week intervals starting one week after transplanting. Sulphur and other appropriate insecticides were used for insect control. Papaya seedlings were field planted when they were about three months old.

FIELD PROCEDURES

Fields were prepared by farm assistants at the station one month prior to planting. Double rows were cut along the contour of the field with 1.8 m (6 ft) spacing between rows and 3.4 m (11 ft) between sets of double rows. Sodium N-Methyldithiocarbamate, a soil fumigant, was injected at a rate of 60 l/ha (40 gal/ac) into each planting row to a depth of eight inches.

Ten days after fumigation, furrows were opened along the fumigated rows and 0.6 m (2 ft) deep planting holes were drilled with a post hole digger every 1.8 m (6 ft). Treble super phosphate at 0.23 kg (1/2 lb) was mixed into each planting hole before the plant was placed. Furrow irrigation was applied as soon as the plants were planted. Two weeks after field planting, 0.23 kg (1/2 lb) of 16-16-16 fertilizer was applied to each tree and 0.45 kg.(1 lb) per tree was applied every 2 months thereafter.

All plants in the study were mechanically inoculated with PRV at 2 to 3 months after transplanting. Fresh virus extract was prepared by macerating one part of young PRV infected papaya leaves in two parts (W/V) of a 0.1 M, pH 7 potassium phosphate buffer plus carbarundum powder. The surface of two fully expanded young leaves on each plant was inoculated by brushing with the plant extract. The inoculated leaves were rinsed with water 5 minutes after inoculation to prevent possible salt damage.

Mosaic mottling of new growth became visible 21 to 28 days after inoculation. Plants that showed no symptoms of infection on the fifth week after inoculation were reinoculated. Severity of infection was evaluated 8 weeks after the first inoculation.

Virus tolerant plants were selected based on the severity of disease symptoms. Hybrids were made by hand pollinating PRV-tolerant females with solo papayas, 'Kapoho solo', 'Waimanalo' and 'Higgins'. Pollinated flowers were protected from pollen contamination with glassine envelopes. Seeds from mature fruits were washed and air dried for further use.

F1 PLANTING, 1982-1983

On March 8, 1982, seedlings from 24 selected papaya lines were planted at the Waimanalo station after a long wet winter. Unfortunately, the bad weather continued and the entire field was destroyed by a rainstorm two weeks after planting. This field was replanted on August 23, 1982 with the same lines except that line 400 was replaced by lines 377 and 378 (Table 2). All trees were mechanically inoculated with PRV on November 16, 1982, and reinoculated in December. Virus infected trees were evaluated for tolerance one month after the second inoculation. Plants were selected based on the severity of disease symptoms. Self-pollinations were made whenever flowers were available.

PARENTAL LINES, F1 AND F2 PLANTING, 1983-1984

In the final planting, 3 solo papaya lines, 'Kapoho solo', 'Waimanalo', 'Higgins'; three 356 lines from sibbings; the F1 plants from crosses between line 356 x 'Kapoho solo' (402), line 356 x 'Waimanalo' (403) and line 356 x 'Higgins' (410), and the F2 plants selected from 2 plants each from 403F1, 402DF1 and 410AF1 were planted in the same field for comparison of PRV tolerance (Table 3). Since 402DF1, 403F1 and 410AF1 lines were produced from different 356 female trees (Table 2), the 356 plants used in this planting were selected especially to match these F1s; for example, line 402DF1 was produced from a cross between 356 R12T8 and 'Kapoho Solo', and the 356 progeny that corresponds to 402DF1 is 356-3 (356 R12T8 x 356 R12T6), which is a half-sibbling of 402DF1.

Table 2--List of F1 hybrids and parental papaya lines planted in field M-1 between August 1982 to August 1983

Line	Female	Male	Sex
356-1	356R3T3	356R3T2	Dioecious
356-2	356R7T19	356R12T6	"
356-3	356R12T8	356R12T6	"
366F3-1	366F2R9T16	366F2R16T25	"
366F3-2	366F2R5T1	366F2R16T35	"
366F3-3	366F2R14T11	366F2R15T20	"
396F1	366F2R5T1	319F3	Hermaphrodite
397AF1	366F2R5T13	356R12T6	Dioecious
397BF1	366F2R14T6	356R12T6	"
397CF1	366F2R5T1	356R12T6	"
397DF1	366F2R14T11	356R112T6	"
398F1	356R7T7	318AF3	Hermaphrodite
400F1	356R7T9	366F2R15T26	Dioecious
401AF1	366F2R5T1	318AF3R3T18	Hermaphrodite
401BF1	366F2R9T16	318AF3R3T18	"
402AF1	356R7T7	Kapoho Solo	"
402BF1	356R7T9	" "	"
402CF1	356R3T3	" "	"
402DF1	356R12T8	" "	"
403F1	356R3T3	Waimanalo	"
410AF1	356R7T7	Higgins	"
410BF1	356R7T9	"	"
Waimanalo			
Kapoho Solo			
377	*C. papaya* 'Thailand' x (*C. papaya* 'Thailand' x *C. cauliflora*) Line 3		
378	*C. papaya* 'Thailand' x (*C. papaya* 'Thailand' x *C. cauliflora*) Line 1		

Table 3--The 3 Hawaiian solo papayas, 3 lines of 356 and the F1, F2 hybrids of 356 x solo papayas planted between November 1983 and September 1984

Line	Female	Male
356-1	356R3T3	356R3T2
Waimanalo	Selfed	
403F1	356R3T3	Waimanalo
403F2A	403F1R13T22 Selfed	
403F2B	403F1R47T12 Selfed	
356-3	356R12T8	356R12T6
Kapoho Solo	Selfed	
402DF1	356R12T8	Kapoho Solo
402DF2A	402DF1R44T23 Selfed	
402DF2B	402DF1R42T24 Selfed	
356R7T7OP.	356R7T7 Open Pollinated	
Higgins	Selfed	
410AF1	356R7T7	Higgins
410AF2A	410AF1R29T24 Selfed	
410AF2B	410AF1R36T28 Selfed	

On November 14 and 16, 1983, 1440 seedlings were transplanted into field M-1, but within 2 weeks, some seedlings in the lower field were lost to root rot diseases. Papaya seedlings in the upper field were not affected by the root rot because this portion of the field was fallowed for 8 months before fumigation. In the lower field, some papaya trees from the previous planting were maintained until late August 1983 for the pollinated fruits. Since fumigants were only applied along the planting rows, the undecomposed papaya stumps between the fumigated areas were probably the source of root rot diseases.

In January 1984, 274 trees in field M-1 were lost to root rot, 159 trees were from replication 4, located in the lower field. All survivors were inoculated with PRV on January 17 and again on February 17, 1984. Plants with high PRV tolerance were selected, and self-pollinations of these plants were made in March and April 1984. The severe drought and reduction in irrigation at Waimanalo beginning in May 1984 resulted in a high incidence of female sterility. No flowers were available for cross-pollinations. Data on tree growth and disease ratings for all the surviving plants were collected between April and August 1984. Statistical analyses were performed on data collected from 3 replications of one-year-old trees.

In this planting, symptoms on virus-infected papayas were classified into six categories: 1) leaf mosaic (LM), 2) leaf distortion (LD), 3) petiole lesions (PL), 4) stem lesions (SL), 5) fruit ringspots (FS) and 6) fruit distortion (FD). The severity of infection was ranked on a scale of 1 to 4, with :

1 = No symptom 2 = Mild symptoms

3 = Moderate symptoms 4 = Severe symptoms (Fig. 1-14).

The cumulative mean (Tmean) was created for each tree by calculating the mean of the 6 symptoms: TMEAN=(LM+LD+SL+PL+FD+FR)/6. The cumulative mean of a tree represents the overall tree response to the virus infection.

The leaf distortion symptom described in this study was not the shoestringing of leaf lobes that was occasionally observed, but the blistering of leaf surfaces and folding of leaf margins.

Other parameters measured included tree height, trunk girth at 30.5 cm (12 in) above ground level, and number of fruits produced. Three to five fruits from each selected tree were measured for weight and total soluble solids content.

This field was planted in a randomized complete block design, with randomization between rows within replications. To conserve planting space, 30 plants from the F1 and F2 lines were planted in each replication, but only 15 plants per replication were planted for the parental lines.

Fig. 1--Disease symptom classification = leaf mosaic (LM)

Severity rating: 1 = no symptom

Fig. 2--Disease symptom classification = leaf mosaic (LM)

Severity rating: 2 = mild

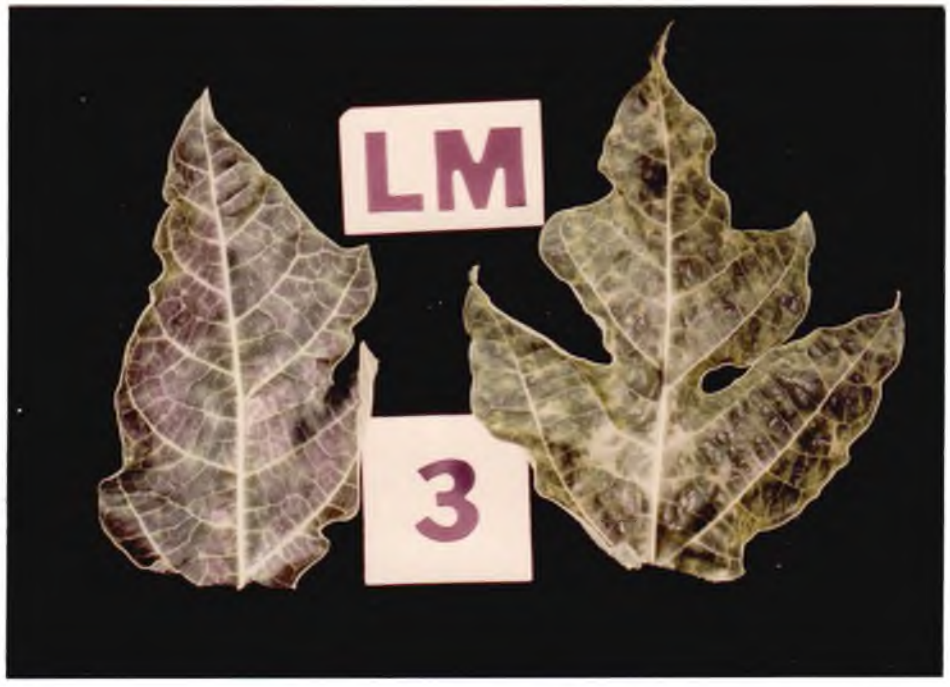

Fig. 3--Disease symptom classification = leaf mosaic (LM)

Severity rating: 3 = moderate

Fig. 4--Disease symptom classification = leaf mosaic (LM)

Severity rating: 4 = severe

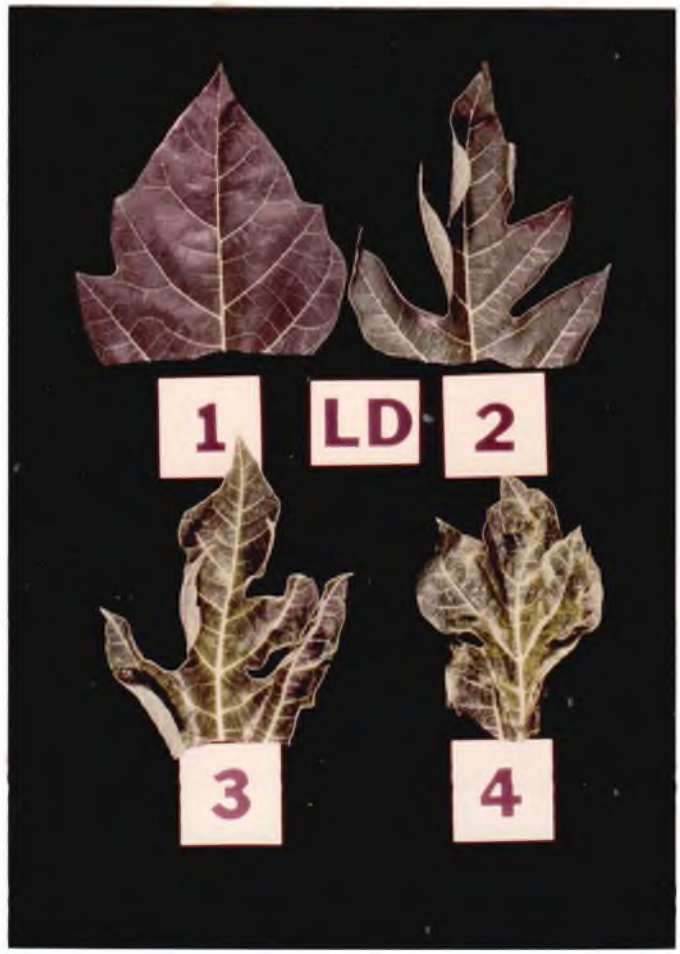

Fig. 5--Disease symptom classification = leaf distortion (LD)

Severity rating: 1 = no symptom 2 = mild

3 = moderate 4 = severe

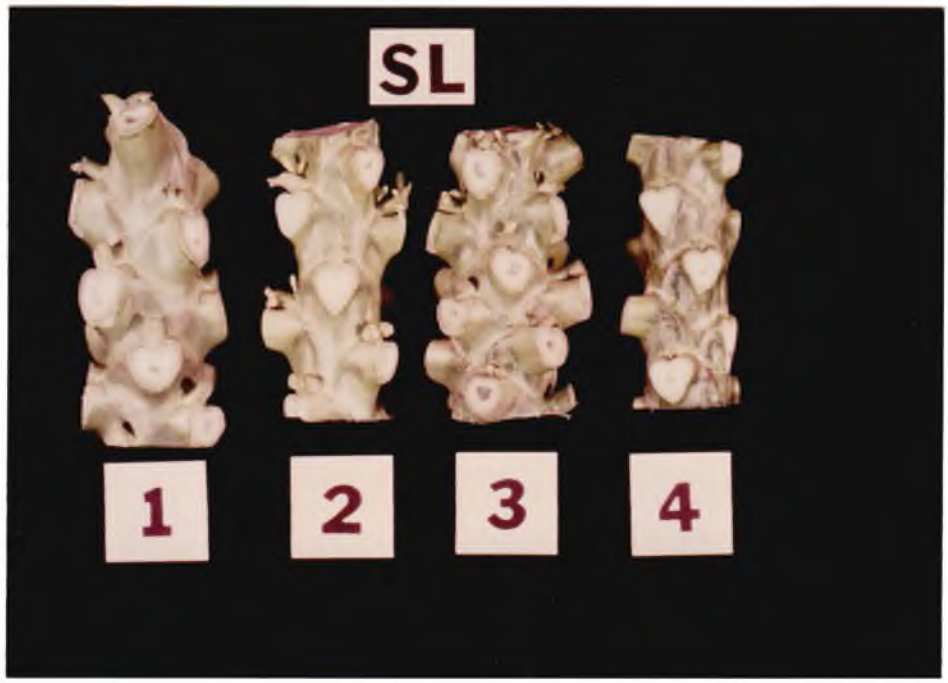

Fig. 6--Disease symptom classification: stem lesions (SL)

Severity rating: 1 = no symptom 2 = mild

3 = moderate 4 = severe

Fig. 7--Disease symptom classification: petiole lesions (PL)

Severity rating: 1 = no symptom 2 = mild

Fig. 8--Disease symptom classification: petiole lesions (PL)

Severity rating: 3 = moderate 4 = severe

Fig. 9--Disease symptom classification: fruit ringspot (FS)

Severity rating: 1 = no symptom

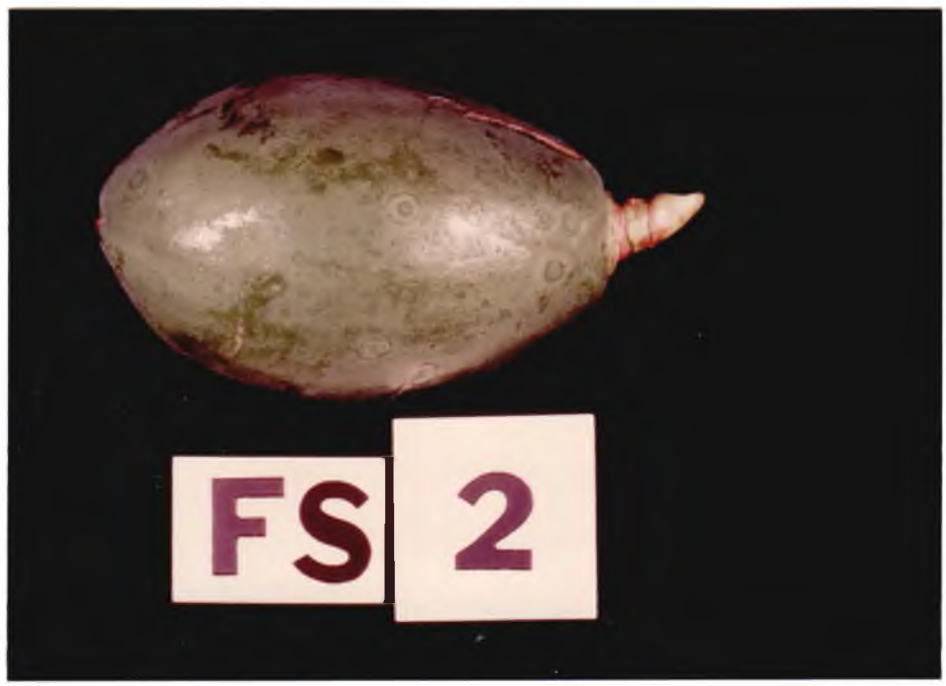

Fig. 10-- Disease symptom classification: fruit ringspot (FS)
Severity rating: 2 = mild

Fig. 11--Disease symptom classification: fruit ringspot (FS)

Severity rating: 3 = moderate

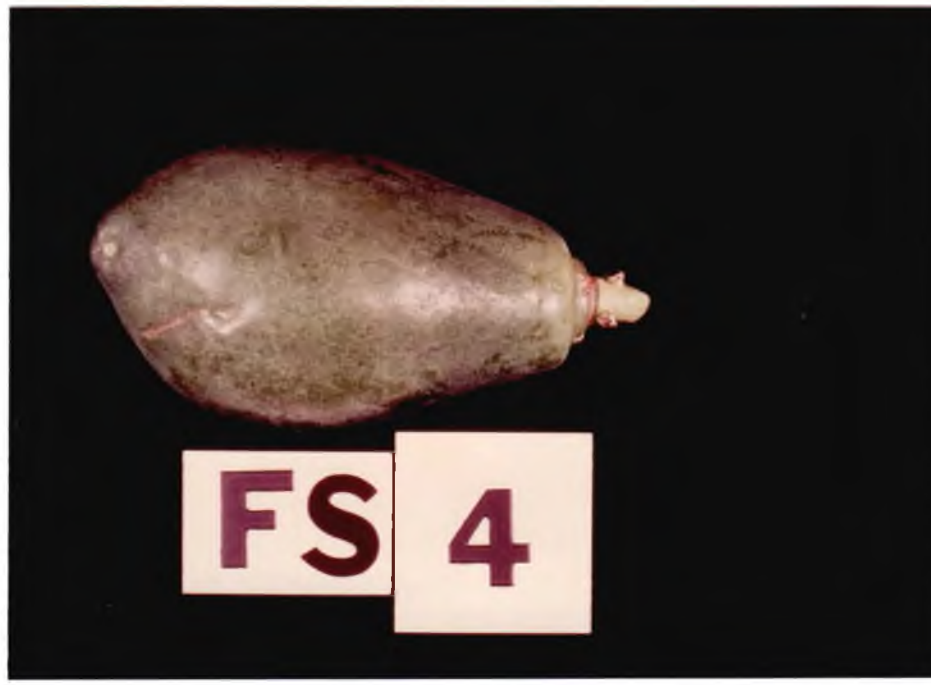

Fig. 12--Disease symptom classification: fruit ringspot (FS)

Severity rating: 4 = severe

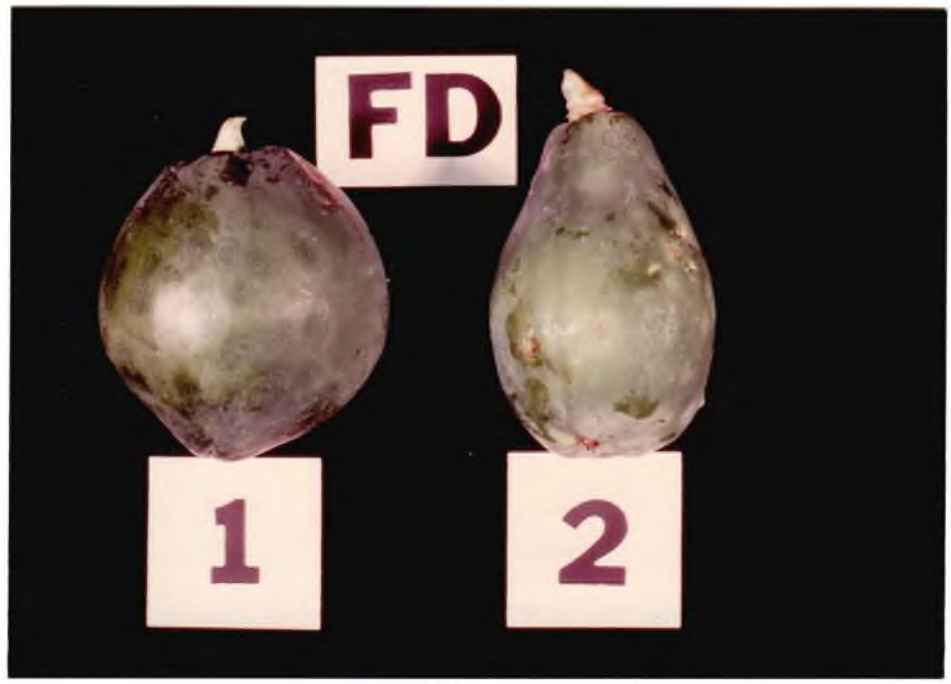

Fig. 13--Disease symptom classification: fruit distortion (FD)

Severity rating: 1 = no distortion 2 = mild distortion

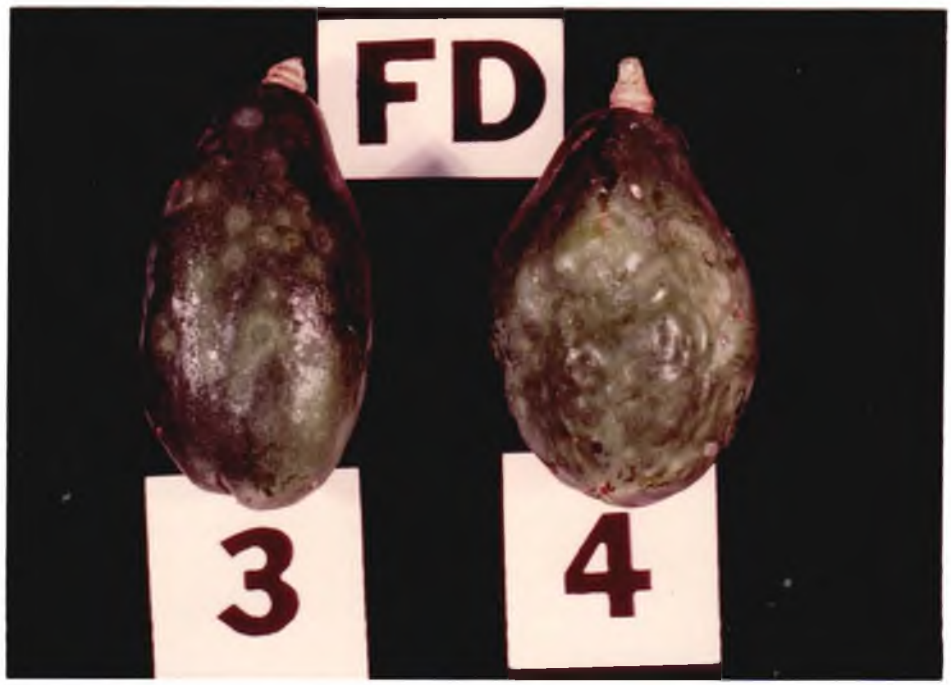

Fig. 14--Disease symptom classification: fruit distortion (FD)

Severity rating: 3 = moderate distortion

4 = severe distortion

INTERSPECIFIC HYBRIDIZATION OF CARICA SPECIES

A separate planting of Carica species was established in the Olinda station on Maui at 1098 meters (3,600 ft.) elevation. The average maximum temperature in August 1982 was recorded at 24°C (75°F) and minimum at 17.8°C (64°F). The mean maximum temperature in November was recorded at 21°C (70°F) and minimum at 16°C (60°F). The purpose of this planting was to determine if cooler temperatures might enhance success in hybridization between C. papaya and other Carica species.

Approximately 0.1 ha was planted with Carica papaya, cultivars 'Kapoho solo' and 'Waimanalo', and Carica species such as C. pubescens (199), C. monoica (312), C. goudotiana (256YF1, 195, 149), C. sphaerocarpa (244), C. microcarpa (197) and a C. cauliflora x C. monoica hybrid (260) of unknown generation. Two replications of 10 plants each were planted in a randomized complete block design. Spacing between plants was 1.8 m (6 ft), and spacing between sets of double rows was 3.4 m (11 ft). Both female and hermaphodite plants of C. papaya were pollinated with C. monoica, C. pubescens, and C. cauliflora x C. monoica (260) on August 25th and October 14th, 1983. All pollinated flowers were protected with glassine envelopes. Fruits were harvested by farm assistants at color break stage and seeds were extracted in the University of Hawaii laboratory.

ENZYME-LINKED IMMUNOSORBANT ASSAY (ELISA)

Enzyme-linked immunosorbant assay (ELISA) is a very powerful technique for the detection and determination of virus infection in Carica papaya. This assay works on the principle of the highly specific antibody-antigen reaction. The ELISA technique was used in this study to determine whether Carica species that failed to produce disease symptoms after repeated inoculation with PRV were truly resistant or were symptomless carriers of the disease.

Carica species, which included C. papaya, C. cauliflora (345, 415), C. pubescens (199), C. stipulata (458, 459), C. quercifolia (450), C. microcarpa (197) and C. pubescens x C. monoica F1 (199x312) were grown in 3-gallon pots in the Magoon Horticultural Facility. They were inoculated with PRV when they were 3 months old and reinoculated at least 5 times during this study. Carica monoica and C. X heilbornii nm. pentagona were planted in the Lyon Arboretum and were included in the study in June 1984. The latter species were inoculated twice. During each series of mechanical inoculations on the species, five C. papaya seedlings were included as a check on the virulence of the PRV source and on the inoculation technique.

A few Carica species, including C. cauliflora (345, 372), C. goudotiana (256), C. quercifolia (450) and C. microcarpa (197) were planted along with PRV-infected papayas at the Waimanalo station in July, 1983. These plants were also assayed for PRV infection with ELISA.

GENERAL ELISA PROCEDURES

Leaf samples from individual plants were harvested on the morning of the assay. A homogenate was prepared for each sample by grinding 6 leaf discs (#4 cork borer) in 1 ml of pH 7.5 buffer containing 0.25M potassium phosphate and 0.01 M EDTA, to make a 1:30 leaf to buffer dilution. The dilution ratio was later changed to 1:50, or 4 leaf discs per ml of buffer.

Cook Micro ELISA Substrate Plates with 96 wells were used in the assay. Individual wells were coated with 200 ul of a solution containing 1 ug/ml of PRV- γ-globulin in pH 9.6 sodium carbonate-bicarbonate buffer. After incubation at 37 C for 2 to 6 hrs, the coating solution was decanted and individual wells on the plate were rinsed 4 times with PBS-Tween buffer (phosphate-buffered saline with 0.05% Tween 20), 3 minutes for each rinse. The wells were then loaded with 200 ul of the homogenate and incubated at 6 C for 18 hours. The plates were decanted after the incubation and individual wells were again rinsed with PBS-Tween before an enzyme-labelled PRV- γ-globulin in a PBS-Tween, 2% polyvinylpyrrolidone (PVP) and 0.2% Ovalbumin buffer at 1 ug/ml was added into the wells. The plate was then incubated at 30 C for 4 hours, emptied, rinsed with PBS-Tween, and 200 ul of p-nitrophenyl phosphate substrate (4 pills per 20 ml) in pH 9.8 diethanolamine buffer was added to each well. If PRV was present in a sample, the contents of that particular well turned yellow within 30 minutes to 1 hour after addition of the substrate. Color intensity was measured with a "Titertek Multiskan" photometer at 405 nm. Details of ELISA procedures can be found in Clark & Adam (1977).

Three hundred microliter of the substrate solution per well was recommended for the final loading of an ELISA plate, 300 ul was the minimum volume recommended to fill the photometer cuvette (Clark & Adam, 1977). A comparison of 12 replications of healthy and virus-infected papaya leaf homogenates were made, using 200 and 300 ul of p-nitrophenyl phosphate substrate.

DETECTION OF INTERFERENCE IN ELISA BY CARICA PLANT HOMOGENATES

Due to the lack of color development in the ELISA of PRV-inoculated *Carica* species, further investigation was conducted to determine if compounds in the leaf homogenates from *Carica* species were interfering with the assay. Leaf samples from uninoculated *C. papaya* 'Kapoho Solo', *C. cauliflora* (345), *C. pubescens* (199) and *C. microcarpa* (197) were collected from the Poamoho Agricultural Experiment Station. A 1:30 leaf to buffer homogenate was prepared by grinding the leaf discs from each of these species with the same amount of leaf discs from a PRV-infected *C. papaya* in pH 7, 0.25M potassium phosphate + 0.1M EDTA. The mixture was then subjected to ELISA for PRV detection. A second treatment was identical to the above, except that the leaf discs of the uninoculated *Carica* species were boiled in water for 5 minutes before being homogenized with the PRV-infected leaf sample.

A related experiment was conducted to determine whether damping of the ELISA reaction by the *Carica* leaf homogenates was due to interference with the PRV- γ-globulin or with the virus itself. A sequential loading procedure was used, in which half of the wells on a γ-globulin coated microplate was first filled with a PRV standard (1:30

dilution), incubated for 4 hours at 37°C, decanted and rinsed as described previously, and then loaded with leaf homogenates (1:30 dilution) from the 4 uninoculated Carica species. The remaining wells on the same plate were loaded in reverse sequence. The PRV standard used in the two treatments was stored at 6°C between the two loadings. All treatments were replicated 3 times on the same micro-plate.

BIOASSAYS FOR PRV IN CARICA SPECIES

All the PRV-inoculated Carica species were assayed for PRV infection using 3-week-old 'Waimanalo' seedlings as indicator hosts. Leaf homogenates from the test plants were prepared by grinding 4 leaf discs (#3 cork borer) in 8 drops (0.5 ml) of pH 7, 0.1 M potassium phosphate buffer. Carbarundum was added to the homogenate and this inoculum was rubbed onto 2 recently expanded leaves with a glass-rod. Inoculated areas were rinsed with water 5 minutes later to prevent possible salt damage.

IV. RESULTS AND DISCUSSIONS

The major objective of this project was to search for PRV-tolerant papayas and to tranfer this tolerance into Hawaiian solo cultivars.

In this study, only selected female trees from the virus-tolerant dioecious lines were crossed with Hawaiian hermaphrodite cultivars. Male plants were not used in order to prevent the introduction of unknown fruit characters in the hybrids.

VIRUS TOLERANCE IN THE PARENTAL LINES

Among the *Carica* species seeds received in 1980, no germination was obtained from *C. cauliflora* (Brazil), *C. pubescens*, or *C. stipulata*. Only *C. cauliflora* (345) from Venezuela and *C. monoica* produced plants. The *C. monoica* plants were highly susceptible to root rot diseases and were lost before they were inoculated with PRV. The *C. cauliflora* (345) plants from Venezuela showed no disease symptoms after repeated inoculations. In 1983, when 6 plants from 2 *C. cauliflora* lines (345, 372) were planted in a field with PRV-infected papayas, no virus symptoms were observed on these trees over a one year period. Bioassays using leaves from the above *C. cauliflora* plants as inoculum did not induce PRV infection on young *C. papaya* 'Waimanalo' seedlings.

All Hawaiian solo papayas were susceptible to PRV. Variation in disease symptoms was observed between cultivars. Upon infection with the virus, 'Waimanalo' plants produced very severe symptoms, with drastic reduction in growth, highly mottled and distorted leaves, abundant stem and petiole lesions, and small, deformed and severely

ringspotted fruits. 'Kapoho Solo' plants were also highly susceptible to the virus. They developed severe symptoms on leaves, stems and fruits, but tree growth was not as seriously retarded as in 'Waimanalo'. Fruit distortion on 'Kapoho solo' was observed to be more severe during cool winter months.

The solo cultivar 'Higgins' was observed to have some tolerance to the virus (Nakasone, Ishii, personal communication), however, this tolerance was not uniformly present in all trees. In the first two plantings (1980-1981, 1982-1983), virus infected 'Higgins' produced disease symptoms as severe as in infected 'Kapoho solo', but in a later planting (1983-1984), a few of the infected 'Higgins' produced only moderate symptoms. Leaf mottling on these trees were relatively mild, with somewhat smooth leaf surfaces and a slight curling of the leaf margins. Few water-soaked lesions were found on stems and petioles. Fruits from these plants were small (0.23Kg) with moderate degree of distortion and ringspotting. These PRV-tolerant 'Higgins' were selfed and seeds were kept for further studies.

Two Okinawa lines, 342 and 343, did not show better virus tolerance than 'Kapoho solo', and were eliminated after the first planting.

The introduction from India, line 366F2, was reported to be a second filial generation of an interspecific hybrid between C. papaya 'Washington' x C. cauliflora. These plants were not immune to PRV but produced moderate to severe symptoms when infected with the virus. Tree morphology varied considerably among these plants; sun-flower type leaves, purple petioles, purple stems and yellow flowers were found as well as normal leaves, green petioles, green stems and white flowers.

From 15 back-crosses made between 366F2 females and C. cauliflora (345), only one fruit was harvested 5 months after pollination. This 3.5 g fruit contained small and aborted seeds.

Leaf samples from 2 to 3 plants each of C. papaya 'Thailand' (83), line 366F3, C. cauliflora (372, 345) and C. papaya 'Thailand' x (C. papaya x C. cauliflora) Line 1 (378) were analyzed by starch gel electrophoresis and zymograms of phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and malate dehydrogenase (MDH) were examined. No difference in isozyme patterns were observed between lines 366F3, 378 and 'Thailand', but these were distinctly different from C. cauliflora isozyme patterns. No evidence suggested that the 366F3 and 378 plants tested were related to C. cauliflora .

FLORIDA PAPAYA SELECTIONS

All 6 Florida introductions were dioecious and were highly susceptible to root rot diseases. Only few plants from line 336, 356 and 357 survived the field planting. The only two surviving plants from line 336 were eliminated from this project due to a lack of PRV tolerance; line 357 was not selected due to poor plant vigor.

Line 356 was the only Carica papaya selection that showed some useful degree of PRV tolerance. Four female and 3 male trees were selected for their mild virus symptoms on fruits, stems and leaves. Upon infection with the virus, plants from line 356 were observed to produce slight chlorosis along the main veins and some upward curling of the leaf margins. Water-soaked lesions were rarely observed on stems or petioles of these trees, and fruits were never distorted. Trees from

line 356 were heterogeneous in tree size, bearing height and fruit qualities (Table 4). Selected female trees from line 356 were crossed with 'Kapoho solo' to produce 402F1, with 'Waimanalo' to produce 403F1, and with 'Higgins' to produce 410F1.

F1 PLANTING IN FIELD M-1, 1982-1983

Lines 377 and 378 were back-crosses of C. papaya 'Thailand' with (C. papaya 'Thailand' x C. cauliflora) F1. When infected with PRV, these plants developed severe leaf symptoms and fruit ringspots. Fruit distortion symptoms were moderately severe. Disease development on line 378 plants was not as severe as on line 377.

When solo papayas were infected with PRV, they produced severe symptoms regardless of the change in environment, however, virus symptoms on the PRV-tolerant 356 lines and their hybrids were observed to fluctuate with environment and tree growth. Virus symptoms on newly infected tolerant plants were somewhat more serious, but gradually, new growth developed milder symptoms. These symptoms remained mild as long as the growing conditions were favorable, but if these plants underwent a period of stress, such as drought or low temperature, an outbreak of more severe symptoms could be expected on the new flush before normal growth resumes.

In 1982, frequent heavy rainfall, hurricane damage and poor root rot tolerance in some of the F1 plants resulted in continuous decline of the field. Virus tolerant trees selected were among the survivors of all these adversities. Line 410AF1, a hybrid of 356 R7T7 x 'Higgins' was observed to have high PRV tolerance. These trees were compact and had

Table 4-- Variations in tree height and fruit quality between selected 356 females 8 months after field planting

Items	356 R3T3	356 R7T7	356 R7T9	356 R12T8
Tree height (meter)	1.53	0.92	1.07	2.14
Bearing ht. (meter)	0.46	0.49	0.61	0.46
Avg. frt. wt. (kg)	1.00	0.82	0.68	1.45
Avg. frt. size (cm)	12 x 12	11 x 11	----	15 x 15
Fruit shape	Round	Round	Round	Round
Ridged	Yes	No	No	No
Flesh color (orange)	Pale	Pale	Pale	Dark
Flavor	Mild	Strong	Strong	Mild
Acidity	No	Slight	No	Moderate
T.S.S.	10.0	11.0	10.8	13.2

medium low bearing height. When infected with PRV, these trees developed a mild degree of leaf mottling, with few water-soaked lesions on stems and petioles, and no fruit distortion. The degree of ringspotting on fruits varied between trees, ranging from a few per fruit to a large number that covered the entire fruit. An upward curling and twisting of leaf lobes was also observed in some of the 410AF1 plants. Among selections from 410AF1, R29T22, R29T24 and R38T14 were the best trees (Table 5, 6).

Hybrids from 356 R3T3 x 'Waimanalo' (403F1) were not as tolerant to the virus as trees from line 410AF1 or line 402DF1. Line 402DF1 was produced from a cross between 356 R12T8 and 'Kapoho Solo' and was anticipated to be the most vigorous and virus tolerant hybrid. Due to hurricane damage, compounded with the root rot problem, only 2 plants from this line were selected (402DF1 R42T24 and R44T23). These trees were vigorous and fast growing, but highly female sterile. Only a few fruits were harvested from these trees. The fruits had mild ringspot symptoms, yellow-orange flesh color, and acceptable fruit qualities. Plants of line 403F1 generally lacked vigor and displayed moderate to severe virus symptoms on leaves, petioles and stems. Fruit production was poor, and average fruit weight was 0.8 kg. Ringspot symptoms on 403F1 fruits ranged from mild to severe, but there was no fruit distortion. Although 403F1 plants were not very vigorous, a few plants were selected because of mild PRV symptoms on fruit, and because of possible Phytophthora root rot tolerance that might have been contributed by the 'Waimanalo' parent.

Table 5-- Values for tree vigor and the severity of virus symptoms on selected 410AF1 plants 8 months after field planting, 1982-83

410AF1	T ht. (cm)	Girth (cm)	Vigor *	Petiole **	Stem	Leaf	Fruit
					symptom ratings		
R29T22	91	20.3	1.0	1	1	2.5	1.0
R29T24	91	20.3	1.5	1	1	3.0	2.0
R36T25	104	20.3	1.5	3	1	3.0	- -
R36T28	102	20.3	1.5	1	3	3.5	2.5
R36T29	127	23.0	2.0	2	1	4.0	1.0
R38T14	114	35.6	1.5	1	1	2.5	2.0

* Tree vigor: 1=vigorous 2=moderate 3=poor 4=dead
** Virus rating 1=no symptoms 2=mild 3=moderate 4=severe

Table 6--Bearing height and fruit characteristics of selected 410AF1 plants 8 months after field planting, 1982-83

410AF1	Bh (cm)	Frtwt (kg)	T.S.S.	Color	Off flavor
R29T22	66.0	0.7	10.5	pale	yes
R29T24	73.7	0.8	13.0	"	no
R36T25	----	0.8	12.9	"	no
R36T28	83.8	0.5	13.5	orange	yes
R36T29	109.2	0.8	13.6	dark or.	no
R38T14	76.2	0.5	14.0	orange	no

In July 1983, 48 plants from 402DF1 were planted along with 11 'Kapoho solo' and nine 356-3 (356 R12T8 X R12T6) in field L-3. These plants were inoculated with PRV in early June and again in August. Data on disease symptom severity were collected in January, 1984. Between July 1983 and January 1984, the growing conditions in Waimanalo were excellent. Rainfall was low, but irrigation was sufficient. All trees grew vigorously and the disease symptoms were serious on infected trees. Infected 'Kapoho solo' trees showed severely mottled leaves, deep oily streaks on petioles and stems, and distorted fruits with severe ringspots.

Plants of line 356-3 were highly tolerant to PRV. Except for occasional chlorosis along the leaf veins and a few ringspots on fruits, these trees were not weakened by the virus disease. Plants from line 356-3 were uniformly tolerant to the virus in this planting.

Disease symptoms on 402DF1 plants were intermediate between line 356-3 and 'Kapoho solo'. Infected 402DF1 trees produced moderately severe leaf mottling, petiole lesions, fruit ringspotting and mild leaf distortion and stem lesions. No distortion of fruit was observed. Virus-infected 402DF1 trees were vigorous, productive and yielded fruits with acceptable eating qualities. Fruits were orange yellow in pulp color with good papaya flavor. Total soluble solids averaged 11% in January 1984, and increased to 16.5 % in some fruits by July. However, these fruits were relatively large (0.8Kg) and had off-shaped seed cavities (Table 7, 8). A slight acid taste was also detected, the higher acid/sugar ratio in these fruits resulted in flavors different from the solo cultivars. This added acid flavor was prefered by some

Table 7--Mean values for virus severity ratings among 11 plants of Kapoho solo, 9 plants of 356-3 and 24 plants of 402DF1 in field L-3, January, 1984

Line	LM*	LD	SL	PL	FS	FD	Tmean
Kapoho	4.0	1.6	4.0	4.0	3.5	2.6	3.3
402DF1	3.1	1.8	2.2	3.0	3.3	1.0	2.4
356-3	2.1	1.3	1.0	1.2	2.0	1.0	1.4

*LM=leaf mosaic, LD=leaf distortion, SL=stem lesions, PL=petiole lesions, FR=fruit ringspots, FD=fruit distortion.
Tmean=mean(LM+LD+SL+PL+FR+FD)
Virus rating 1=no symptoms 2=mild 3=moderate 4=severe

Table 8--Mean values for average tree height, tree girth, bearing height, fruit count, fruit weight between 9-month-old Kapoho solo, 402DF1 and 356-3, January 1984

Line	Tht (cm)	Tg (cm)	Bht (cm)	Frt#	Frtwt (kg)	T.S.S.
Kapoho	188	35	123.9	20.3	0.23	12.5
402DF1	175	39	61.5	34.1	0.79	11.2
356-3	109	30	31.2	28.7	0.90	9.4

Table 9--Virus rating on selected 402DF1 trees in field L-3, 1983-1984

ID	LM	LD	SL	PL	FS	FD	Tmean
R2T3	3*	1	3	2	2.4	1	2.07
R2T10	3	2	3	2	3.3	1	2.38
R2T11	3	2	3	2	2.4	1	2.23
R2T20	3	2	3	2	2.5	1	2.25
R4T5	3	1	2	2	2.1	1	1.83
R4T6	3	1	2	3	3.0	1	2.17

* Virus rating 1=no symptoms 2=mild 3=moderate 4=severe
LM=leaf mosaic LD=leaf distortion SL=stem lesions PL=petiole lesions
FS=fruit ringspots FD=fruit distortion

Table 10--Tree height, girth, bearing height, fruit count, fruit weight and total soluble solids of selected 402DF1 trees in field L-3,1983-1984

ID	Tht (cm)	Tg (cm)	Bht (cm)	Ft#	Fwt (kg)	T.S.S. winter	T.S.S. summer
R2T3	206	45.7	73.2	30	0.75	11.5	16.5
R2T10	193	38.1	71.0	29	0.55	10.8	-- --
R2T11	203	43.2	63.5	34	0.64	11.4	-- --
R2T20	175	35.6	76.2	30	0.92	10.6	14.0
R4T5	198	40.6	76.2	30	0.92	10.6	15.5
R4T6	196	43.2	73.7	34	0.74	11.0	14.5

Fig. 15-- The PRV-tolerant papaya hybrid line 402DF1 and the susceptible cultivar 'Kapoho solo' in field L-3, 8 months after infection with PRV

and disliked by others. Six 402DF1 trees from this planting were selected and self pollinated for F2 seeds (Table 9, 10).

COMPARISONS BETWEEN PARENTAL LINES, F1 AND F2

The papaya lines in this planting are arranged in Tables 11 and 12 according to the cumulative mean of disease ratings for the 6 symptom categories. Mean ratings for the individual symptom categories, as well as a mean vigor rating are given for each line. 'Higgins' plants were observed to be more tolerant to PRV in every symptom category than trees from 'Waimanalo' or 'Kapoho Solo'. Average cumulative mean value for 'Higgins' was 2.66 which was significantly lower in the overall disease severity than 'Waimanalo' (Tmean= 3.90) and 'Kapoho Solo' (Tmean= 3.88) (Table 12).

Trees from Lines 403F1, 403F2A and 403F2B were more susceptible to PRV than those from lines 402 F1, F2s, and lines 410AF1 and F2s. The stem lesions and fruit ringspots were more severe than other disease symptoms in the 403 lines.

The average rating for stem lesion in line 410AF2B was 2.11, which was not significantly different from 356-1 (2.06) or 356-3 (2.03). No significant difference was observed in fruit distortion ratings between the 356 lines and most 356 x solo papaya hybrids. 'Higgins' and 403F2A were rated mild in the fruit distortion symptom, 'Waimanalo' and 'Kapoho solo' were rated severe (Table 13, 14).

Trees from 402 lines were significantly more vigorous than most of the 410 or 403 lines. Average tree height for 402DF1 , 402DF2B, 402DF2A was 182.6 cm, 173.8 cm and 159.8 cm respectively. No significant

Table 11-- Mean values for severity rating of leaf mosaic, leaf distortion, stem lesion, and petiole lesion symptoms on 3 Hawaiian solo cultivars, 356, F1 and F2 hybrids of 356 x solo papayas 6 months after infection with Papaya Ringspot Virus

Lines	Leaf mosaic	Leaf dist	Stem lesion	Petiole lesion
Waimanalo	3.95a*±0.22	3.85a*±0.43	3.95a*±0.22	3.97a*±0.16
Kapoho Solo	3.98a±0.15	4.0a ±0	4.0a ±0	4.0a±0
403F2A	3.29b±0.68	3.12bc±0.88	3.52b±0.68	3.28b±0.83
403F1	3.22b±0.68	2.94bc±0.75	3.67b±0.51	3.31b±0.66
403F2B	3.25b±0.69	2.85bc±0.77	3.59b±0.69	3.21b±0.83
Higgins	3.09bc±0.45	3.15b±0.50	2.59de±0.56	2.24d±0.50
402DF2B	2.75d±0.63	3.05bc±0.74	3.05c±0.83	2.58c±0.73
410AF1	2.86cd±0.65	2.86bc±0.60	2.61de±0.79	2.61c±0.73
402DF1	2.62d±0.60	2.86bc±0.68	2.77d±0.68	2.62c±0.66
402DF2A	2.76d±0.72	2.97bc±0.70	2.45e±0.66	2.54c±0.70
410AF2B	2.74d±0.59	3.03bc±0.66	2.11f±0.31	2.05de±0.27
410AF2A	2.68d±0.55	2.83c±0.58	2.37e±0.51	1.97de±0.43
356R7T70P	2.30e±0.59	2.48d±0.62	2.39e±0.61	2.09de±0.45
356-3	1.89f±0.56	2.28de±0.53	2.03f±0.42	1.66f±0.48
356-1	1.94f±0.68	2.19e±0.66	2.06f±0.57	1.81ef±0.75

*Mean separation in column by Duncan Multiple Range Test, 5% Level
Virus rating 1=no symptoms 2=mild 3=moderate 4=severe

Table 12--Mean values for severity rating of fruit ringspots and fruit distortion, cumulative means (Tmean) and tree vigor for 3 Hawaiian solo cultivars, 356, F1 and F2 hybrids Of 356 x solo papayas 6 months after infection with Papaya Ringspot Virus

Lines	Ft.Rings*	Ft.Distort	Tmean	Tree Vigor**
Waimanalo	3.83a±0.38	3.61a±0.97	3.90a±0.25	2.97a±0.35
Kapoho Solo	3.59ab±0.61	3.47a±0.87	3.88a±0.20	2.26bc±0.49
403F2A	3.31bc±0.75	2.06b±1.33	3.17b±0.69	2.47b±0.78
403F1	3.35bc±0.63	1.10c±0.47	2.94c±0.38	2.11cd±0.80
403F2B	3.19bcd±0.72	1.33c±0.84	2.92c±0.49	2.15bc±0.79
Higgins	3.00cde±0.83	1.74b±1.16	2.66d±0.42	2.29bc±0.57
402DF2B	2.78efg±0.81	1.18c±0.65	2.59d±0.47	1.53fgh±0.68
410AF1	3.27bcd±0.59	1.00c±0	2.53de±0.37	2.06cde±0.75
402DF1	3.09cde±0.65	1.04c±0.25	2.51de±0.37	1.26h±0.56
402DF2A	2.42gh±0.66	1.09c±0.53	2.39ef±0.42	1.63fg±0.81
410AF2B	2.82efg±0.78	1.02c±0.14	2.33f±0.30	2.12cd±0.85
410AF2A	2.89def±0.71	1.04c±0.36	2.31f±0.31	1.81def±0.82
356R7T70P	2.53fg±0.64	1.13c±0.52	2.24f±0.36	1.36gh±0.60
356-3	1.90i±0.57	1.00c±0	1.91g±0.33	1.59fgh±0.78
356-1	2.10hi±0.74	1.00c±0	1.89g±0.47	1.75ef±0.77

Mean Separation in column by Duncan Multiple Range test, 5% level
* Virus rating 1=no symptoms 2=mild 3=moderate 4=severe
** Tree vigor rating 1=vigorous 2=moderate 3=poor 4=dead

Table 13--Mean values for tree height, tree girth, fruit count and fruit weight on 3 Hawaiian solo cultivars, 356, F1 and F2 hybrids of 356 x solo papayas 6 months after infection with Papaya Ringspot Virus

Line	Tree ht. (cm)	Tree girth (cm)	Frt. count	Av.frt.wt. (kg)
Waimanalo	97.9h±21.8	13.7e±3.4	3.92d±5.1	0.20
Kapoho Solo	173.5a±26.5	22.5bcd±5.4	10.6c±9.8	0.10
403F2A	123.4fg±38.7	18.7de±8.3	9.38c±11.4	0.57
403F1	144.5cd±26.4	24.9abc±6.6	15.9ab±9.8	0.66
403F2B	145.8cd±29.6	19.7cd±5.7	11.6bc±8.6	0.35
Higgins	121.8fg±29.2	18.4de±5.5	12.9abc±11.4	0.21
402DF2B	173.8a±38.8	27.2ab±7.3	17.6a±12.3	0.42
410AF1	141.7cde±28.3	24.5abc±7.2	16.6a±9.8	0.46
402DF1	182.6a±30.2	28.2a±8.4	16.4a±12.1	0.47
402DF2A	159.8b±35.6	25.8ab±7.1	17.3a±10.4	0.40
410AF2B	134.3de±26.5	21.6bcd±6.7	9.96c±8.9	0.41
410AF2A	135.4de±31.2	25.5abc±7.6	16.3a±11.3	0.41
356R7T70P	149.1bc±24.6	28.7a±4.81	8.00cd±10.2	0.78
356-3	129.0efg±27.3	24.0abcd±6.1	4.86d±7.4	0.54
356-1	116.3g±35.5	20.2cd±6.8	11.1c±12.5	0.49

Mean saparation in column by Duncan Multiple Range test, 5% level

Table 14--Values for range on fruit weight, total fruit weight per tree, total soluble solids content and bearing height on 3 Hawaiian solo cultivars, 356 and F1, F2 hybrids of 356 x solo papayas 6 months after infection with Papaya Ringspot Virus

Lines	Range of wt. (kg)	Av.tot.wt. (kg)	T.S.S	Bearing ht (cm)
Waimanalo	- - - - -	0.78	14.0	74.3
Kapoho Solo	0.09-0.27	1.06	15.0	147.3
403F2A	0.09-1.36	5.35	13.0	63.8
403F1	0.36-1.14	10.5	13.5	65.5
403F2B	0.19-0.68	4.06	12.0	72.6
Higgins	0.16-0.23	2.71	13.0	66.7
402DF2B	0.13-0.82	7.39	12.8	94.2
410AF1	0.27-0.61	7.64	13.1	57.4
402DF1	0.20-1.00	7.71	15.5	85.2
402DF2A	0.20-0.68	6.92	14.5	90.7
410AF2B	0.21-0.73	4.08	14.6	61.3
410AF2A	0.16-0.73	6.68	10.9	63.9
356R7T70P	0.16-1.18	6.24	10.5	52.9
356-3	0.30-0.77	2.62	13.0	50.7
356-1	0.32-0.68	5.44	10.5	47.6

difference was observed between tree girth measurements among Line 402DF1, 356R7T70P, 356-3, 402DF2A, 410AF1, 402DF2B and 403F1. Plants from 356-1, 356-3, 403F2A and 'Higgins' were more compact; 'Waimanalo' trees were severely stunted (Table 13, 14).

Seventeen individual trees were selected from this planting. Among the selected plants, 2 trees were from line 403F2, 8 trees from 402DF2, 6 plants from 410AF2 and one plant from 410AF1 (Table 15, 16). The two selected 403F2 trees were 403F2A R1T10 and 403F2B R13T7; 403F2A R1T10 was vigorous, with mild leaf and moderate stem and petiole symptoms. There was a moderate amount of ringspot on fruits, but there was no distortion. Flesh was bright orange yellow in color and the fruit had a large seed cavity. Total soluble solids averaged 14.5%, but fruits had open blossom ends, soft flesh and an unpleasent after-taste.

Tree 403F2B R13T7 was tall and vigorous with mild leaf symptoms. Petiole lesions and fruit ringspots were moderate, but the tree had severe stem lesions. Fruits were firm with uniform seed cavity and orange yellow in flesh color. Total soluble solids was 13.2% and had a slight acid taste.

Eight plants from 402DF2 were selected. Plant 402DF2A R7T1 was vigorous with mild leaf distortion and fruit ringspot. Leaf mottling and stem lesions were rated moderate, but petiole lesions were very severe. This plant had short, upright leaf petioles.

Tree 402DF2B R19T21 was vigorous with high virus tolerance, but fruit production was poor. Only 3 fruits were harvested from this plant and they never softened, even when fully colored. Average total soluble solids content was 13.5%. These fruits had strong papaya odor.

Table 15-- Values for severity ratings of leaf mosaic, leaf distortion, stem lesions, petiole lesions, fruit ringspots, fruit distortion and cumulative mean in selected PRV-tolerant papayas 6 months after infection with PRV.

Tree	Virus ratings*						
	LM	LD	SL	PL	FS	FD	Tmean
402DF2							
R7T1	3	2	3	4	2	1	2.5
R7T6	3	4	2	2	2	1	2.3
R19T2	3	3	2	3	2	1	2.3
R19T21	2	2	2	2	2	1	1.8
R22T8	3	3	2	2	2	1	2.1
R22T14	2	2	2	2	2	1	1.8
R22T17	3	2	2	3	2	1	2.1
R22T30	3	2	3	2	3	1	2.3
403F2							
R1T10	2	2	3	3	3	1	2.3
R13T7	2	2	3	4	3	1	2.5
410AF2							
R11T9	2	3	1	2	2	1	1.8
R11T18	3	3	2	2	2	1	2.2
R26T5	2	2	2	2	3	1	2.0
R26T17	2	2	2	2	2	1	1.8
R33T9	2	3	2	2	2	1	2.0
R33T10	2	2	2	2	2	1	1.8
410AF1							
R18T17	2	2	2	2	3	1	2.0

* Virus ratings 1=no symptoms, 2=mild, 3=moderate, 4=severe
LM=leaf mosaic, LD=leaf distortion, SL=stem lesions, PL=petiole lesions
FS=fruit ringspots, FD=fruit distortions, Tmean=cumulative mean.

Table 16-- Tree vigor, bearing height and fruit characteristics of selected PRV-tolerant trees 6 months after infection with PRV

Line	Tv*	Tht (cm)	Tg (cm)	Bh (cm)	#Frt	Frtwt (kg)	T.S.S
402DF2							
R7T1	1	193	27.9	88.9	20	0.49	13.0
R7T6	1	152	35.6	81.3	20	0.68	13.0
R19T2	1	175	38.1	86.4	40	0.54	14.5
R19T21	1	221	38.1	94.0	3	0.68	13.5
R22T8	1	180	38.1	58.4	24	0.56	14.5
R22T14	1	196	27.9	111.8	25	0.40	16.3
R22T17	1	172	30.5	66.0	32	0.28	15.5
R22T30	1	229	38.1	111.8	40	0.42	16.8
403F2							
R1T10	1	160	25.4	76.2	17	0.56	14.5
R13T7	1	190	25.0	58.4	20	0.40	13.0
410AF2							
R11T9	1	152	35.6	81.3	12	0.34	10.9
R11T18	1	147	38.1	60.9	16	0.38	10.7
R26T5	1	216	40.6	50.8	42	0.45	13.1
R26T17	1	127	33.0	50.8	28	- - -	- -
R33T9	1	152	35.6	60.0	14	- - -	- -
R33T10	1	158	22.9	65.0	9	0.45	14.0
410AF1							
R18T17	1	132	35.6	53.3	32	0.55	14.0

*Tree vigor ratings 4=dead 1=vigorous

Trees 402DF2 R19T2, R22T17,and R22T30 were selected trees that were productive and tolerant to PRV infection. Fruits from 402DF2 R19T2 had the best seed cavities among all 402DF2 trees. The cavities were uniformly compact and seeds could be removed easily. Average total soluble solids content was 14.5%. Fruits from 402DF R19T2 had slightly acid flavor. Trees R22T17 and R22T30 were tall and vigorous, R22T30 was highly productive with total soluble solids averaged 16.8%. The total soluble solid for R22T17 averaged 15.5% (Table 15, 16).

Two plants were selected from line 410AF2A in March, and 4 more trees were selected in August, 1984. These trees were shorter than those selected from line 402DF2. Poor fruit production and quality were the major problems in this line. Most of the selected 410AF2 trees had very mild virus symptoms on leaves, stems and fruits. Fruits from 410AF2 R11T9 were elongated (16.5 x 6.35 cm) with total soluble solids of 10.4%. Fruits from R11T18 were also elongated (14 x 8.3cm), with pale flesh color and undesirable after-taste. Total soluble solids content averaged 10.5%. The most productive tree was 410AF2 R26T5. Fruits were firm, with mild flavor but with a slightly bitter after-taste. Total soluble solids content averaged 13%. Tree 410AF1 R18T17 was compact, vigorous and productive. Fruits were firm, with orange yellow flesh. Total soluble solids content averaged 14% (Table 15, 16).

Pollinations should be made between 410AF2 R26T5, R11T9, R11T18 with 402DF2 R19T2, R22T14, R22T30, and the 402DF1 trees selected from field L-3 (Table 9, 10). These selected 402 and 410 trees will

complement each other in the improvement of both fruit qualities and virus tolerance.

FREQUENCY DISTRIBUTION FOR SEVERITY IN PLANT SYMPTOMS

Frequency distributions of disease severity ratings were calculated for each of the 6 symptom categories and the cumulative means (Tmean) to show the improvement in virus tolerance of the 356 x solo papaya hybrids relative to the susceptible solo parents (Fig 16-22). However, no valid genetic analyses could be obtained from these frequency distributions because 1) lines 402DF1, 403F1 and 410AF1 were produced from different dioecious female trees selected from the non-inbred line 356; the 356 lines used in this planting were half sibblings of the F1s, which may be different from the maternal parents in virus tolerance; 2) the F2 lines in this planting were derived from 2 selected trees each from lines 403F1, 402DF1 and 410AF1 (Table 3), and were not random F2 populations.

The PRV tolerant papayas were selected based on the severity of disease symptoms. Since the fruit is the most important product of papaya plants, selection for trees with mild fruit symptoms was emphasized over other symptom categories; trees that produced fruits with severe ringspots were usually discarded regardless of the severity of other symptoms, but trees that produced fruits with mild fruit symptoms were retained if the other symptoms were not severe. This preferential selection resulted in a relative lack of improvement in leaf symptoms in the F2 populations (Fig 17, 18).

Except for the fruit distortion symptom, disease severity in each symptom category appeared to be quantitatively inherited. Most of the

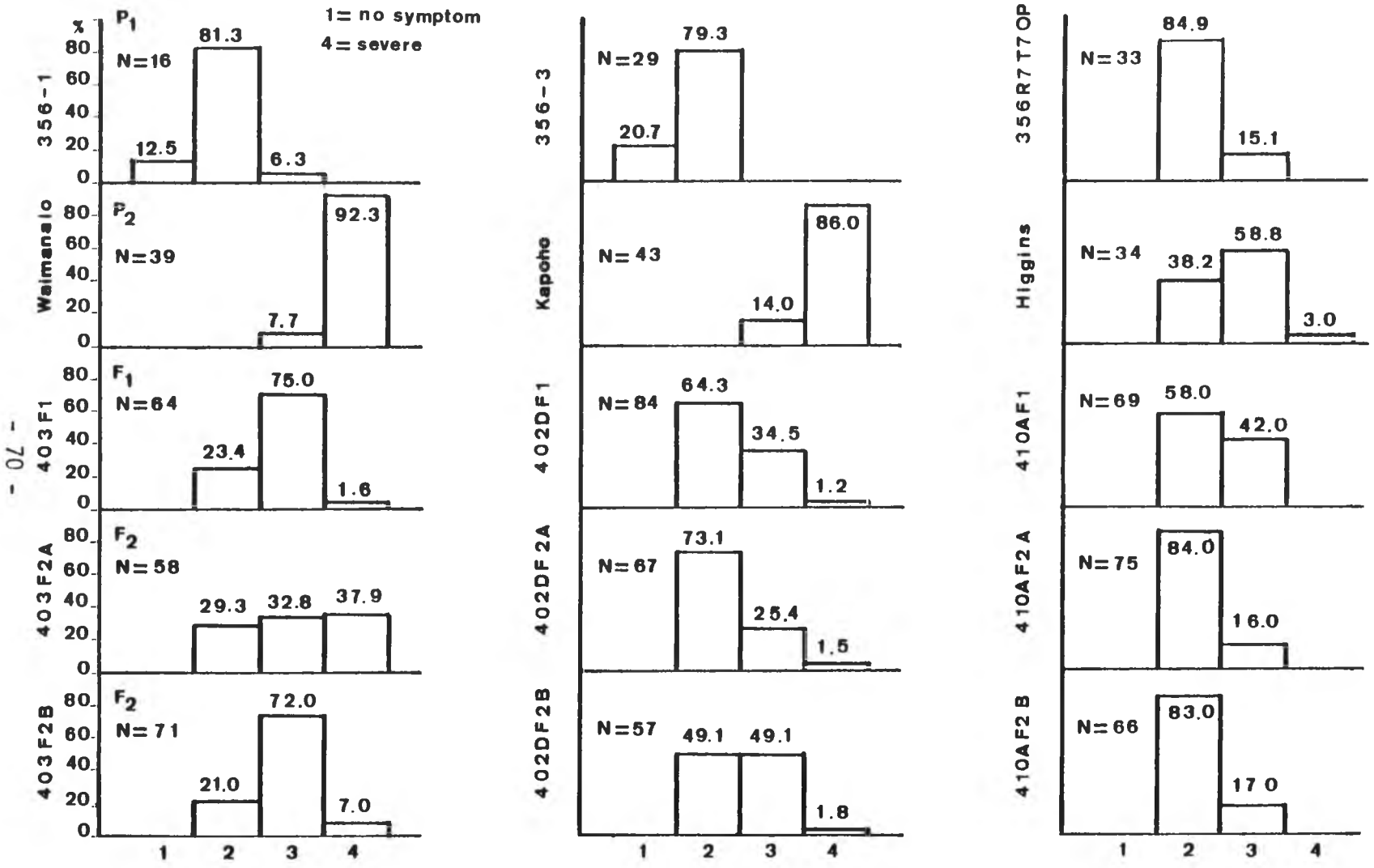

Fig.16-- Frequency distributions in percent, for 'Cumulative Means' (Tmean) of disease symptom severity rating for PRV-infected solo papayas, 356 and 356 x solo hybrids in field M-1, 1984.

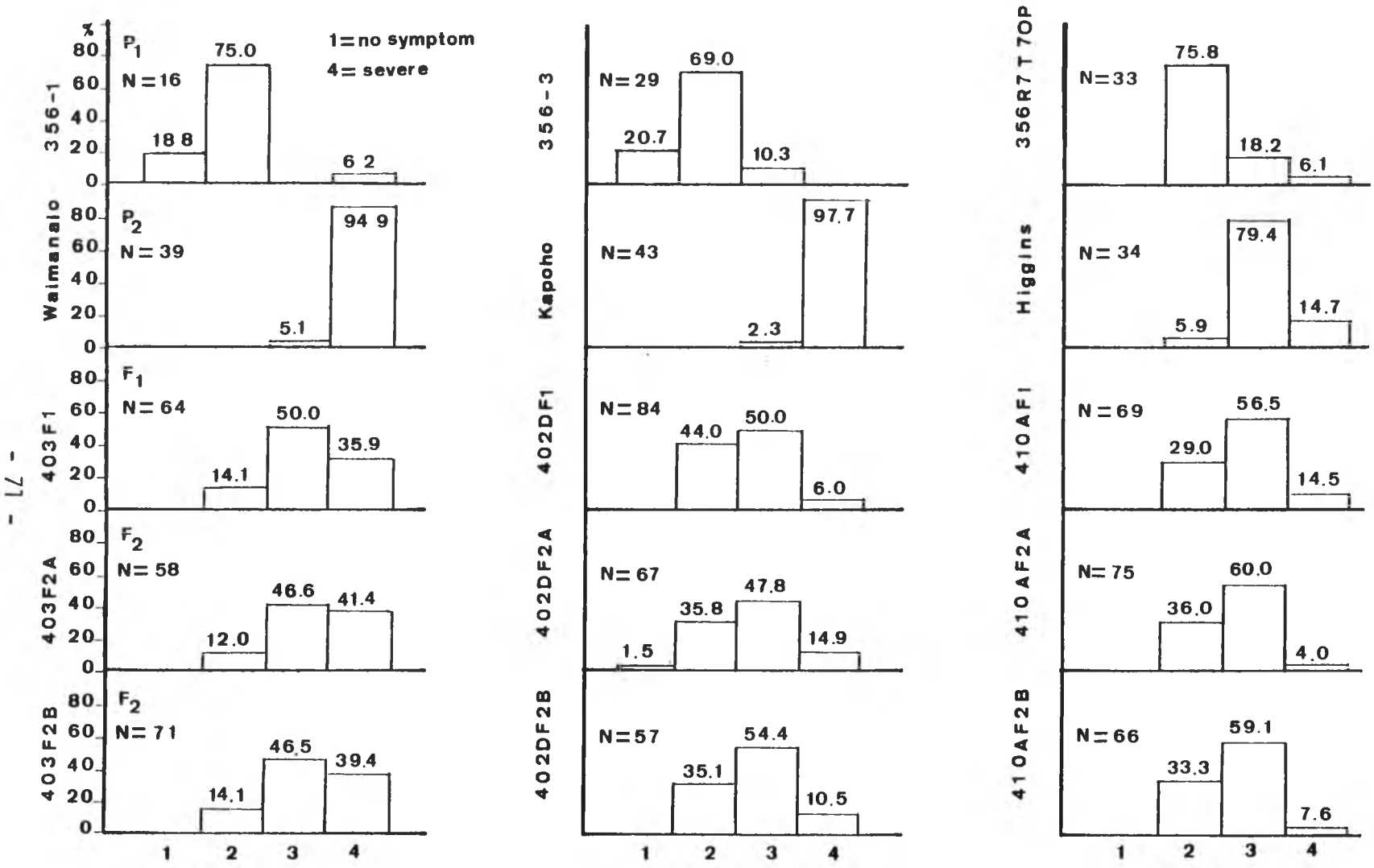

Fig.17-- Frequency distributions in percent, for severity ratings of 'Leaf Mosaic' symptoms for PRV-infected solo papayas, 356 and 356 x solo papaya hybrids in field M-1,1984.

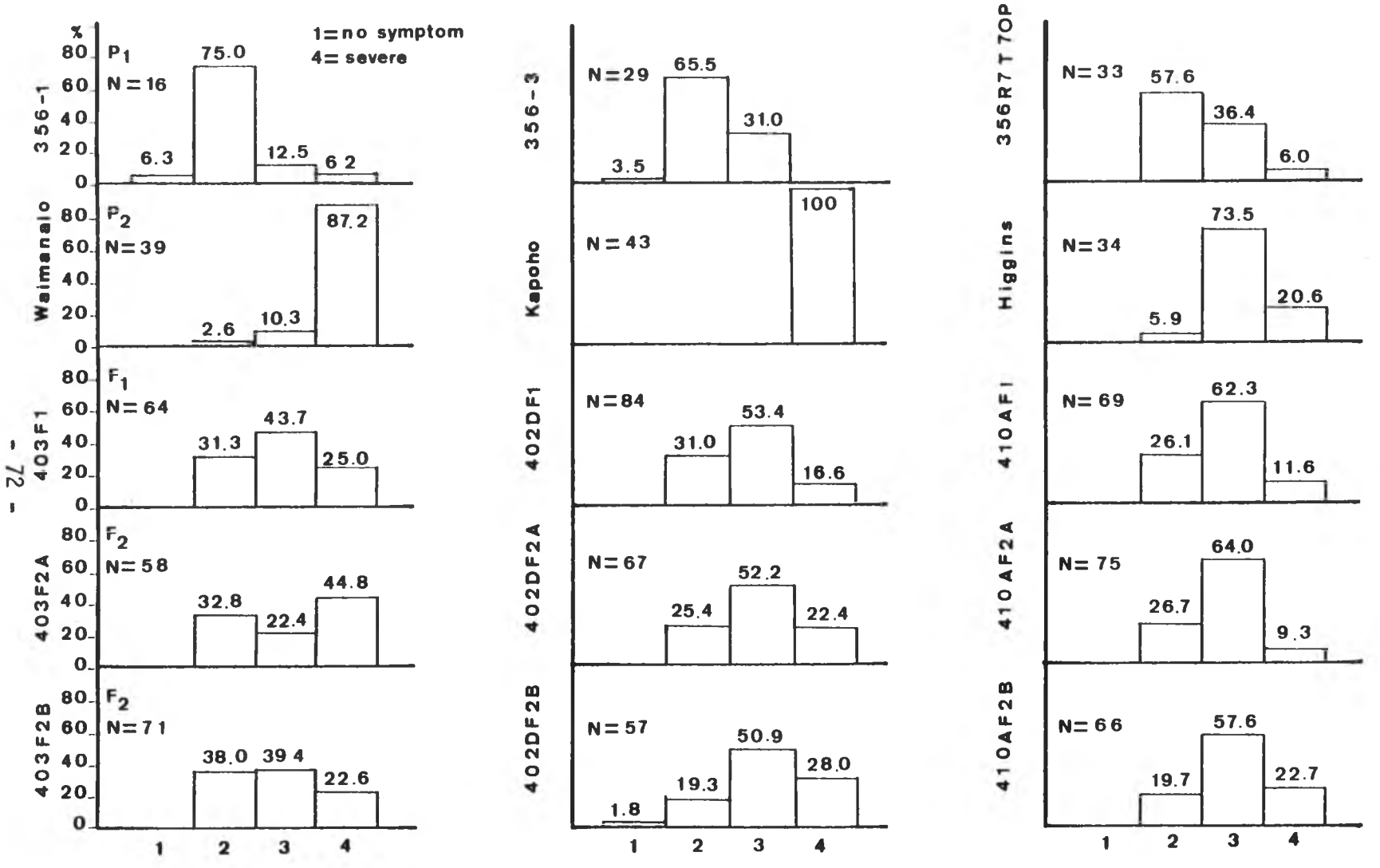

Fig.18-- Frequency distributions in percent, for severity ratings of 'Leaf Distortion' symptoms for PRV-infected solo papayas, 356 and 356 x solo papaya hybrids in field M-1,1984.

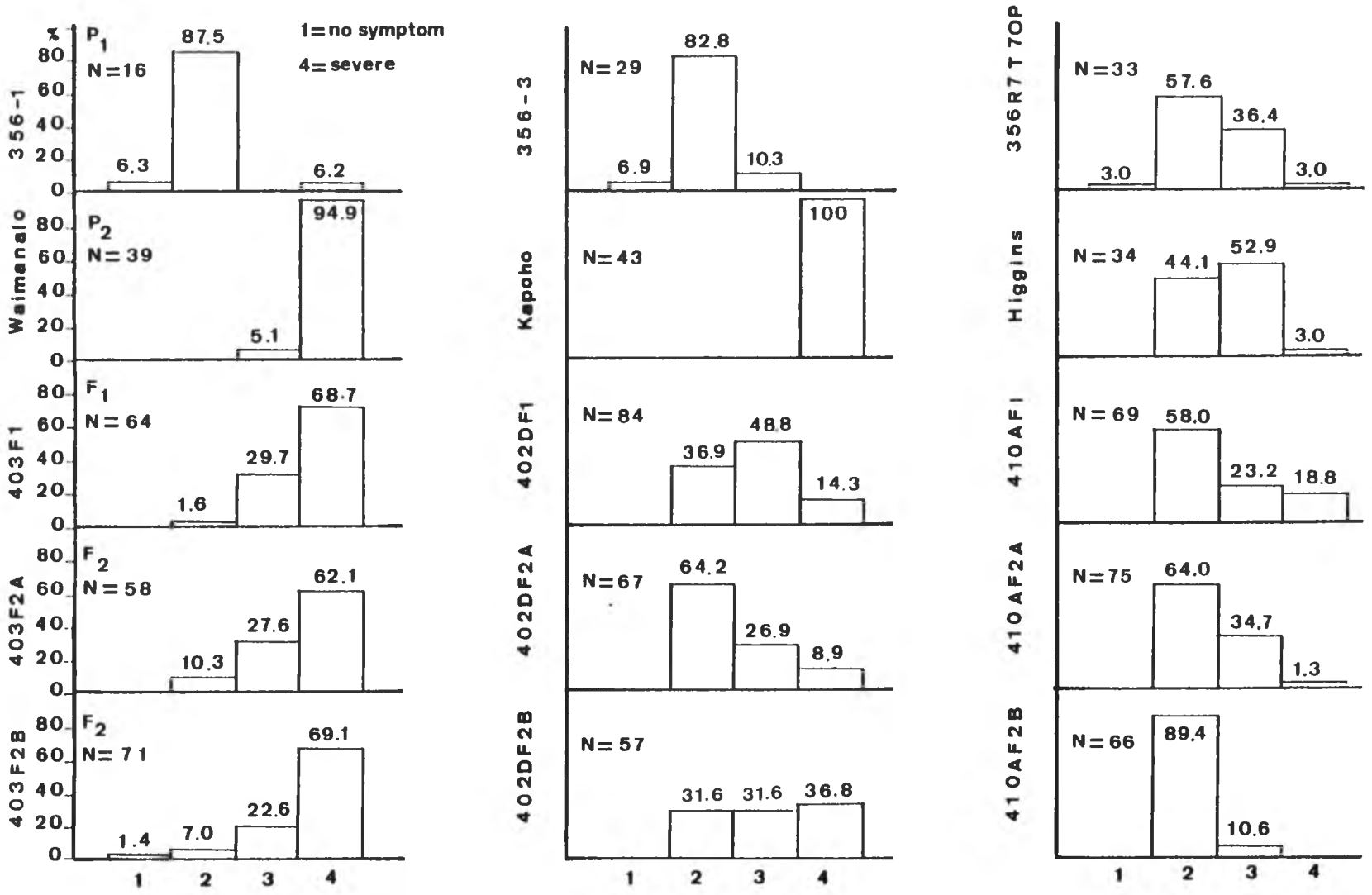

Fig.19-- Frequency distributions in percent, for severity ratings of 'Stem Lesion' symptoms for PRV-infected solo papayas, 356 and 356 x solo papaya hybrids in field M-1, 1984.

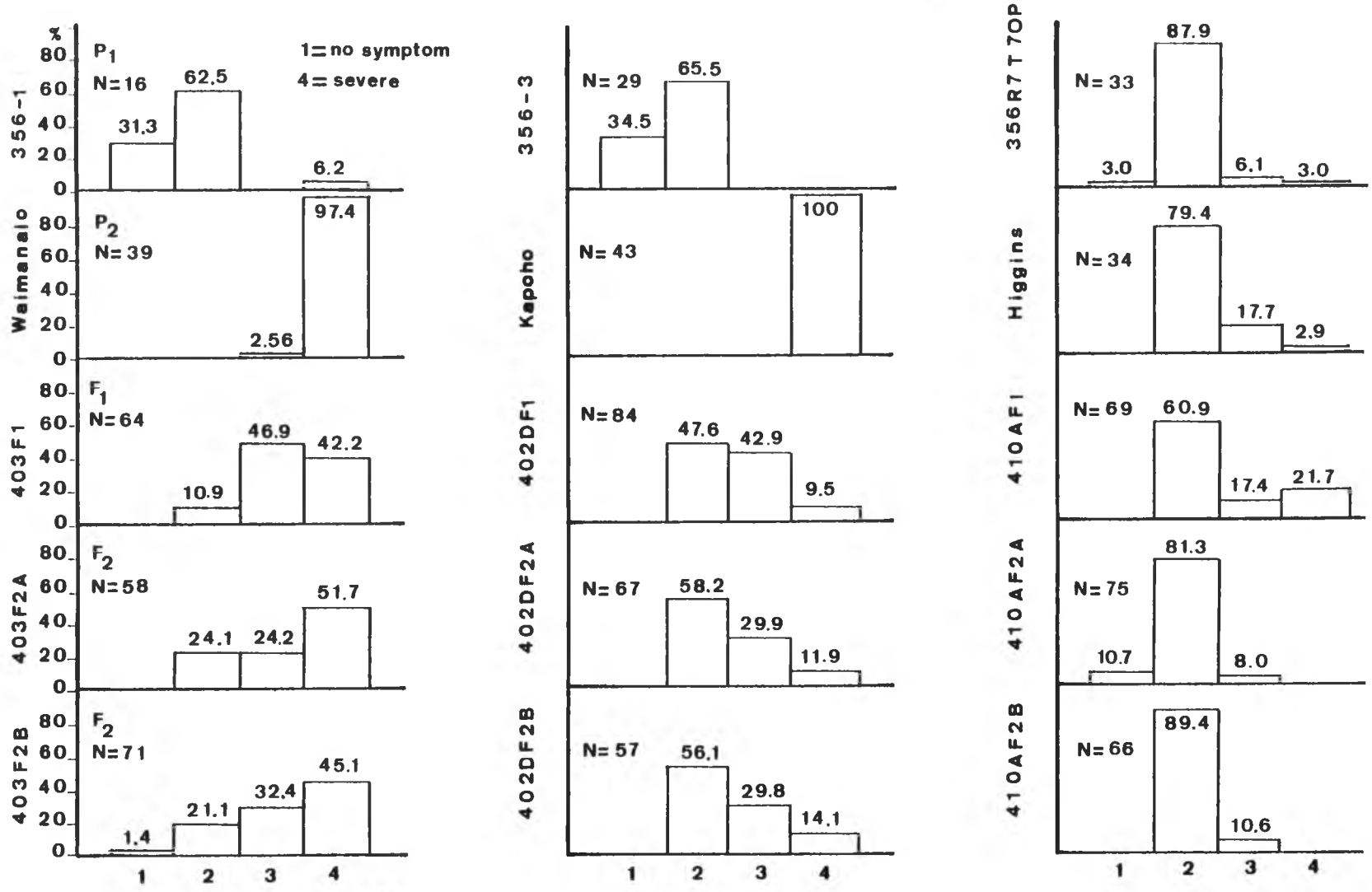

Fig.20--Frequency distributions in percent, for severity ratings of 'Petiole Lesion' symptoms for PRV-infected solo papayas, 356 and 356 x solo papaya hybrids in field M-1, 1984.

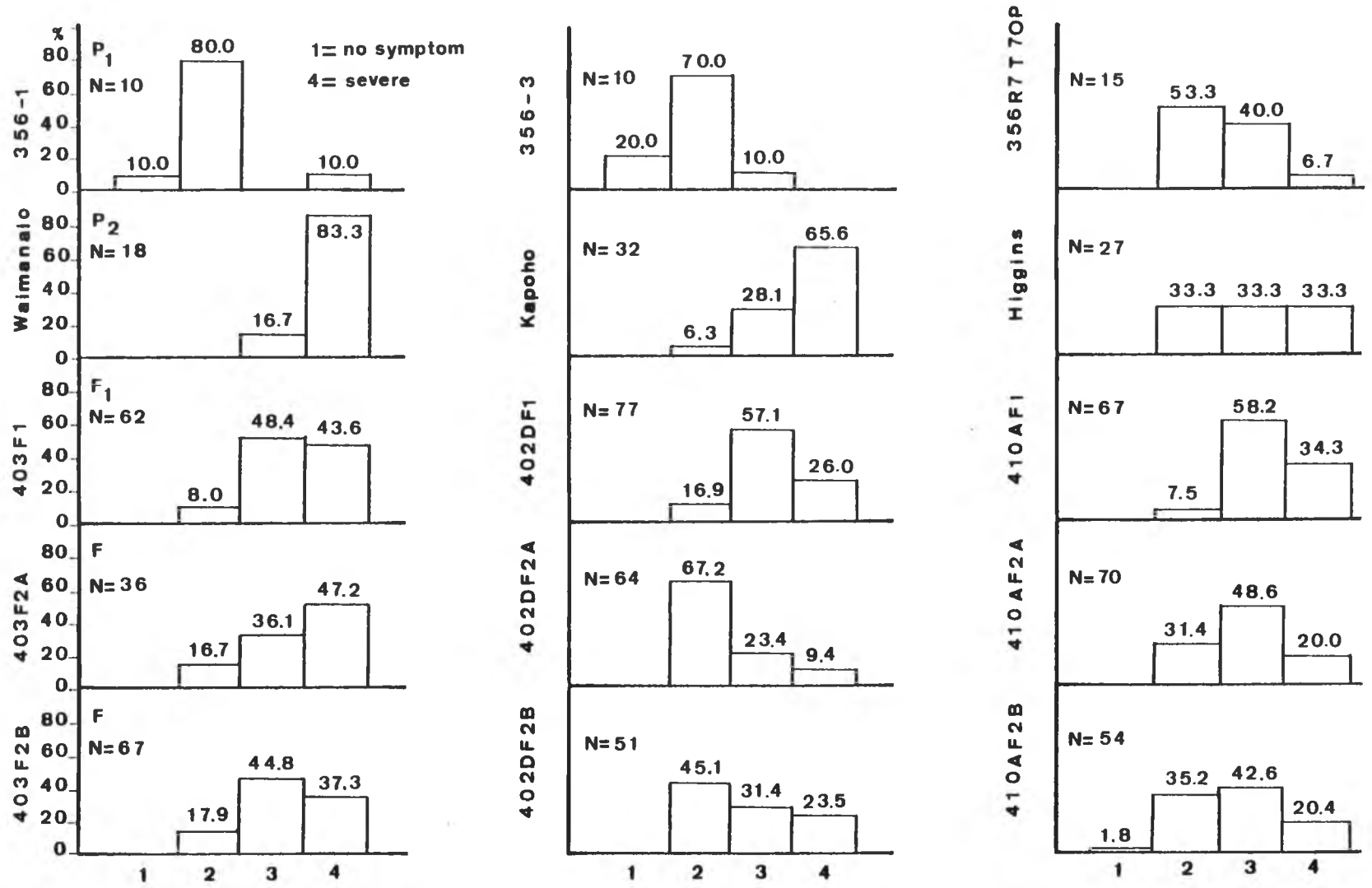

Fig.21-- Frequency distributions in percent, for severity ratings of 'Fruit Ringspot' symptoms for PRV-infected solo papayas, 356 and 356 x solo papaya hybrids in field M-1, 1984.

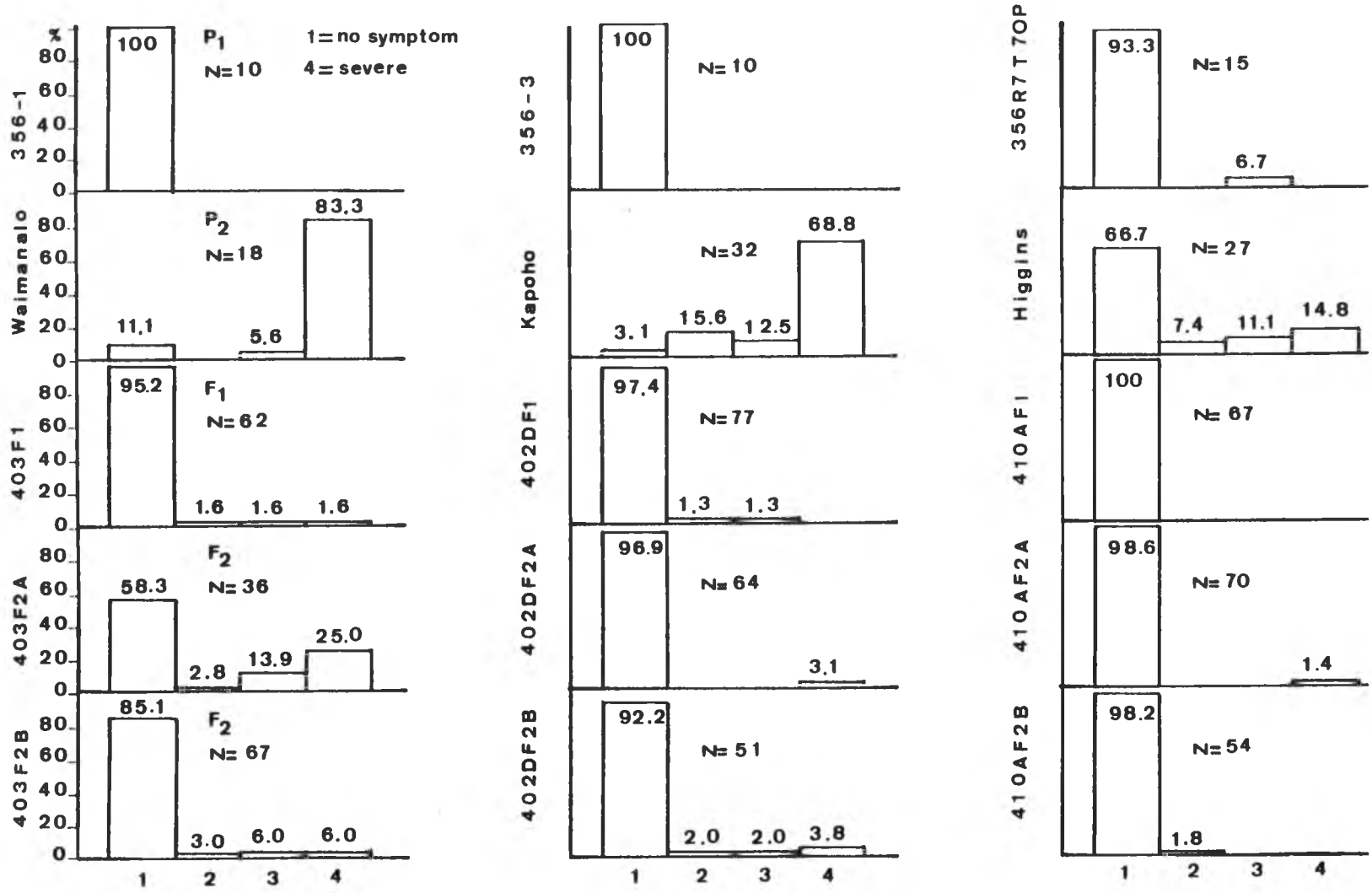

Fig.22-- Frequency distributions in percent, for severity ratings of 'Fruit Distortion' symptoms for PRV-infected solo papayas, 356 and 356 x solo papaya hybrids in field M-1, 1984.

356 x solo papaya hybrids were intermediate in symptom severity between the tolerant and susceptible parents, but most of the stem and petiole symptoms from the trees from line 403F1 and the derived F2s were rated severe. The majority of 'Higgins', 410AF1 and 410AF2s had moderate stem and mild petiole lesion symptoms (Fig 19, 20).

Fruit distortion was never observed in fruits of line 356 and rarely in 356 x solo papaya hybrids. Fruit distortion in solo papaya lines appeared to be a recessive character that was suppressed in crosses with line 356 as the female parent. More distorted fruits were observed in trees from line 403F1 and F2 than in other hybrid lines (Fig 22).

CORRELATION IN DISEASE SEVERITY BETWEEN SYMPTOMS

When all the parental, F1 and F2 lines were analyzed as a group, significant negative correlation (1% level) was observed between the cumulative means (Tmean) and the rating for tree vigor (r=-0.41), tree girth (r=-0.12), and number of fruits produced (r=-0.09). Significant correlation was also observed in the symptom severity between leaf mosaic and leaf distortion, leaf mosaic and petiole lesions, stem lesions and petiole lesions, stem lesions and fruit ringspots in lines 402F2, 403F2 and 410F2 when the F2 plants in each of these crosses were combined and analyzed as a group. The symptom severity in leaf mosaic and stem lesions, leaf distortion and stem lesions, leaf distortion and petiole lesions, leaf distortion and fruit ringspots, petiole and fruit ringspot, leaf mosaic and fruit distortion, and fruit ringspots and fruit distortion were significantly correlated in 2 out of the 3 F2

populations. There was no correlation between stem lesions and fruit distortion in all the F2 lines (Table 17).

CONTROL OF PRV THROUGH CROSS-PROTECTED VIRUS-TOLERANT PAPAYAS

A virus-tolerant papaya line should have all the desirable qualities of a commerical cultivar. This can be achieved in time through breeding but until PRV-tolerance is bred into the commercial lines, PRV-tolerant papayas should not be grown along with susceptible cultivars. Since PRV can multiply in the tolerant papaya lines, using these plants indiscriminately may result in the unintentional spread of the virus.

Under greenhouse conditions, a mild strain of PRV, HA 5-1, successfully cross-protected papaya seedlings from challenge inoculations with PRV HA. However, "superinfection" was observed when very young leaves or the whole plant of a cross protected plant were inoculated with PRV HA. "Superinfected" plants produced severe virus symptoms within 1 to 2 months (Yeh & Gonsalves, 1984).

Papaya Ringspot Virus may be controlled by using cross-protected PRV-tolerant papayas. The assumption is that if large number of papayas cross-protected with a mild strain of PRV are released continuously by the University or a commercial source, there is a good chance that the mild strain will be spread by aphids, slowly diluting the severe strain of PRV in the wild. However, in order for the public to accept these cross-protected plants, a virus-tolerant papaya cultivar should be used. The genetic tolerance will prevent cross-protected plants from showing

Table 17--Coefficients of correlation between symptoms of PRV in *Carica papaya* lines in 402DF2, 403F2, and 410AF2.

	F2	LM	LD	SL	PL	FS
LD	402	0.32**				
	403	0.51**				
	410	0.32**				
SL	402	0.24**	0.25**			
	403	0.46**	0.43**			
	410	0.11	0.02			
PL	402	0.23**	0.26**	0.47**		
	403	0.55**	0.46**	0.69**		
	410	0.25**	0.06	0.25**		
FS	402	0.21	0.32**	0.39**	0.25**	
	403	0.39**	0.39**	0.43**	0.40**	
	410	0.17	0.07	0.33**	0.15	
FD	402	0.32**	0.31**	0.12	0.13	0.29**
	403	0.34**	0.23	0.18	0.22	0.48**
	410	0.06	0.02	0.19	0.25**	0.18

LM= leaf mosaic, LD= leaf distortion, SL= stem lesions, PL=petiole lesions, FS= fruit ringspots, FD=fruit distortion.

** significant at 1% level.

severe virus symptoms even when superinfected with the severe strain of PRV.

INTERSPECIFIC HYBRIDIZATION BETWEEN CARICA SPECIES

Crosses between C. cauliflora and 'Kapoho Solo' were made during 1980-1981. Female trees of C. cauliflora (345) pollinated with C. papaya produced normal sized fruits, but had no viable seeds. Reciprocal crosses using hermaphodite papayas as the female were not successful. During 1982-83, fruits were produced on every peduncle of a female C. cauliflora planted in a field of C. papaya but no viable seed was obtained.

At the Olinda station in Maui, female and hermaphrodite flowers of 'Kapoho solo' and 'Waimanalo' were pollinated with C. pubescens (199), C. monoica (312) and a C. cauliflora x C. monoica hybrid (260)of unknown generation. About 50% fruit set was obtained from each of these crosses. Fruit size ranged from 40 g for fruits in crosses between 'Kapoho solo' x C. monoica (312) and 'Kapoho solo' x C. pubescens (199) to the two 500 g fruits from crosses between 'Waimanalo' and the cauliflora x monoica hybrid (260).

In January 1983, 4 fruits each of 'Kapoho solo' x 199 and 'Waimanalo' x 199 were harvested at the Olinda station 3 months after pollination. Immature seeds of less than 2 mm were aseptically extracted and cultured in a medium composed of 1/2 M.S. salts, 30g/l sucrose, 9g/l agar, iron chelate, 100mg/l myo-inositol, 1 mg/l thiamine, 1 mg/l nicotinic acid and 1/2 mg/l pyridoxin. Four out of 15 seeds from

'Kapoho solo' x 199 turned green after 3 weeks in culture, but developed no further. No growth was detected from the seeds of 'Waimanalo' x 199.

No seed was found in 6-month-old fruits of 'Kapoho solo' x 260, but about 20 to 30 seeds were extracted from each of the 'Waimanalo' x 260 fruits. These seeds were dark to light brown in color and about half the size of the normal 'Waimanalo' seeds. No germination was obtained from seeds collected from 8 different 'Waimanalo' x 260 fruits.

One 'Kapoho solo' x 312 fruit weighing 350 g, was harvested 10 months after pollination and 190 dark, well developed seeds were extracted. Two out of 8 seeds germinated and produced papaya-like seedlings. Further observations will be needed to determine the authenticity of these hybrids.

Papaya seeds harvested from the Olinda station generally had poor germination. No germination was obtained from samples of 'Kapoho solo' and 'Higgins'. Other Carica species seeds harvested from the station germinated fairly well.

Cool temperature enhanced the growth and development of the Carica species studied, but did not increase the success in interspecific hybridization between C. papaya and other Carica species. However, most of these fruits remained on the trees for the full 5 months and some had normal sized fruits. The low temperature significantly delayed fruit maturation in Carica papaya. Self-pollinated fruits remained on the trees for as long as 9 to 12 months. Some of these fruits decomposed at color-break stage.

Some successful interspecific crosses made in the same period were C. pubescens x C. monoica (199 x 312) and reciprocal; C. microcarpa x

C. monoica (197 x 312) and reciprocal; C. goudotiana x C. microcarpa (256YF1 x 197) and C. goudotiana x C. pubescens (149 x 199). Two plants from C. goudotiana x C. microcarpa (256YF1 x 197) were vigorous, both trees had white freckle-like markings on stems and petioles as did C.goudotiana, and white callus-like growth near the base of each petiole as did C. microcarpa. One of these hybrids had reddish color on the stem and petioles.

PRV In Carica Species

During the early stage of this study, leaf homogenates from virus infected Carica species were prepared by grinding 6 leaf discs (#4 cork borer) in 1 ml of extraction buffer. The buffer was composed of pH 7.5, phosphate-buffered saline (PBS), 0.05% Tween-20, 2% polyvinyl pyrrolidone (PVP-40) and 0.2% ovalbumin. Using this extraction procedure, only PRV-infected C. papaya samples yield positive results in ELISA, and color intensities were different between replications. The color was more intense if the PRV-infected papaya homogenates were agitated by repeated pumping of the pipette before transferring into individual wells, and color development in samples that were not agitated was much milder. This extraction buffer was later replaced by pH7, 0.25M potassium phosphate buffer containing 0.1M EDTA. The new buffer produced more intense and consistent ELISA results with PRV-infected papayas.

Carica monoica and C. X heilbornii were included in PRV ELISA's and bioassays since June 1984. Plants of C. X heilbornii and all solo papayas showed positive infection symptoms.

The first symptoms on C. X heilbornii were observed 3 weeks after inoculation. The plant developed moderate chlorosis and severe vein-clearing on recently matured leaves, with blistering and puckering of the young growth. Water-soaked lesions were found on the stem 6 weeks after inoculation. Leaf homogenates prepared from infected C. X heilbornii induced a very light color reaction in ELISA (Table 18). Bioassays on 6-week-old 'Waimanalo' seedlings resulted in 3 transmissions out of the 6 plants inoculated; infection symptoms on the

papaya seedlings appeared 28 days after inoculation. Inoculation controls, using PRV-infected 'Waimanalo' leaf as the inoculum source, caused infection in 4 out of 4 'Waimanalo' seedlings within 10 days.

Carica monoica was reported to be highly susceptible to PRV and other diseases (Nakasone, personal communication). One C. monoica plant in the Lyon Arboretum was inoculated with PRV on June 22, 1984, along with a C. X heilbornii. The latter tree produced visible disease symptoms within 3 weeks, but no infection was observed on the C. monoica. This plant was reinoculated on October 3, 1984, and vein clearing symptoms on new leaves were observed 28 days later. However, ELISA results on this tree were negative. Bioassays using PRV inoculated C. monoica leaves as the inoculum source did not cause virus infections in 5 'Waimanalo' seedlings inoculated.

Carica pubescens, C. quercifolia, and C. stipulata were highly susceptible to mite damage. Mite induced leaf distortions on these plants resembled PRV leaf distortions in C. papaya, however, ELISA results on the distorted leaf samples were negative.

Carica cauliflora (345) produced no virus symptoms after inoculation with PRV. 'Waimanalo' seedlings inoculated with C. cauliflora leaf homogenates did not develop virus symptoms. One C. cauliflora (415) plant was observed to develop mosaic mottlings along the leaf veins, but ELISA results were negative (Table 18).

In July 1983, C. cauliflora (372- 4 plants), (345- 1 plant), C. goudotiana (256- 3 plants), C. microcarpa (197- 1 plant) and C. quercifolia (450- 4 plants) were planted along with PRV infected C. papaya cv. Kapoho, 402DF1 and 356-3. These plants were inoculated when

Table 18-- Mean values of color intensity in Enzyme-Linked Immunosorbant Assay (ELISA) of *Carica* species for Papaya Ringspot Virus

Species	Inoculated E405	Uninoculated E405
C. papaya	1.758	0.117
C. X *heilbornii* nm. *pentagona*	0.219	0.142
C. cauliflora (345)	0.147	0.122
" " (415)	0.101	0.119
C. pubescens	0.101	0.093
C. stipulata	0.099	0.111
C. quercifolia	0.097	0.102
C. monoica	0.096	0.101
C. microcarpa	0.094	0.097
C. pubescens x *C. monoica*	0.094	0.109

[a] Average values from 3 replications.

they were 3 months old and were continuously exposed to the natural virus challenge. No virus symptoms were observed on the wild Carica species, although the C. papaya lines were uniformly infected. Bioassays using papaya seedlings as indicators with leaf homogenates from PRV-inoculated C. cauliflora (345, 372) and C. quercifolia (450) as inoculum resulted in no virus transmission. Carica goudotiana and C. microcarpa were lost before a bioassay could be conducted.

In October, 1984, 3-week-old 'Waimanalo' seedlings were used as host plants for PRV-bioassays for C. goudotiana (256), C. quercifolia (450), C. cauliflora (345, 415), C. pubescens (Volcano), C. stipulata (459), C. microcarpa (197), C. monoica (312), C. heilbornii n.m. pentagona, C. papaya cv. 'Waimanalo', C. pubescens x C. monoica (199 x 312) F1 and C. goudotiana x C. microcarpa (256 x 197) F1. Three out of 5 'Waimanalo' seedlings inoculated with PRV-infected C. heilbornii developed mosaic mottlings on leaves 4 to 5 weeks after inoculations. Fourteen out of 16 'Waimanalo' seedlings inoculated with PRV-infected C. papaya developed severe leaf symptoms within 3 weeks after inoculations. No other PRV-inoculated Carica species transmitted the virus disease to the C. papaya hosts.

Using 200 ul of p-nitrophenyl-phosphate substrate solution in the final loading of ELISA plates resulted in significantly lower extinction values (E405) than the 300 ul loaded wells. The average value for 12 replications of healthy papaya samples was 0.113 for the 200 ul loaded wells and 0.151 for the 300 ul. The average E405 value for PRV-infected papaya was 0.882 for the 200 ul and 0.972 for the 300 ul loaded wells. These differences in the extinction values were not

due to differences in the availability of substrate to the enzymes, but rather to the different pathlengths of light through the substrate solution.

The E405 values should be interpreted as relative, rather than as absolute indicators of the amount of virus present in samples.

INTERFERENCE WITH ELISA RESULTS BY CARICA SPECIES LEAF COMPONENT

When equal numbers of PRV-infected papaya leaf discs were homogenized with leaf discs from a healthy C. microcarpa or C. pubescens plant to make a 1:30 dilution, the virus in the mixture was not detectable by ELISA. However, when the virus infected leaf discs were homogenized with leaf discs from C. cauliflora or C. papaya, only a slight reduction in color intensity was observed (Table 19). This inhibition of ELISA was largely overcome when the leaves from C. microcarpa or C. pubescens were boiled in hot water for 5 minutes before being homogenized (Table 19). No logical explanation could be given for the unanticipated reduction in color intensity when the boiled C. papaya and the PRV standard were mixed.

The purpose of having the different loading sequence was to show that, if the interference to the ELISA was due to the binding of the leaf homogenate to the γ-globulin, wells that were filled with leaf homogenates before the PRV standard would develop no color reaction. If the interference was due to the binding of Carica leaf components to the virus particles, the ELISA reaction in wells that were first filled with PRV standard and then with the leaf homogenate would not develop more color than wells that were filled with leaf homogenates before the virus

Table 19--Effects of uninfected Carica species leaf homogenates and heat-killed Carica leaf homogenates on the development of color intensity in ELISA detection of Papaya Ringspot Virus.

	Treatment	
	Papaya Ringspot Virus infected leaf discs +	
	Healthy leaf discs E405	Boiled leaf discs E405
C. papaya	0.931	0.668
C. cauliflora (345)	0.757	0.811
C. pubescens	0.072	0.759
C. microcarpa	0.076	0.568

Average values from 3 replications.

E405 for PRV-infected papaya at 1:30 dilution = 0.862

E405 for healthy C. papaya = 0.037

E405 for healthy C. pubescens = 0.027

E405 for healthy C. microcarpa = 0.026

E405 for healthy C. cauliflora = 0.031

standard. No positive ELISA reaction was observed in wells that were filled in the sequence of C. microcarpa or C. pubescens and then the PRV standard. Only slight virus-induced color reactions were observed in wells that were first loaded with C. papaya or C. cauliflora and then the PRV standard (Table 20). Since mixing healthy C. papaya or C. cauliflora leaf homogenate with PRV standard did not reduce extinction values in the previous experiment (Table 19), the reduction in color intensity in wells sequentially exposed to leaf extracts of healthy C. papaya or C. cauliflora and then to PRV standards was unexpected.

A hypothesis was developed from these observations. It was hypothesized that a certain cell component in all the Carica species interferes with PRV detection in ELISA. The concentration of this component varies in different Carica species and is high in C. pubescens and C. microcarpa but low in C. papaya and C. cauliflora. When PRV standard was mixed with leaf homogenate from C. microcarpa or C. pubescens and assayed by ELISA, the "interference component" (IC) from these species quickly attached to the antibodies because of the high concentration, and no virus-antibody reactions could take place.

When the same PRV standard was mixed with leaf homogenate from C. papaya or C. cauliflora, the virus-induced color reaction was not significantly affected, since the concentration of the "IC" in these species is not great enough to be competitive with PRV for the antibodies.

In the sequential loading experiment, antibodies in wells that were first filled with C. microcarpa or C. pubescens leaf homogenate, were quickly sequestered by the "interference component" and were not

Table 20--Detection of interference effects on Enzyme-Linked Immunosorbant Assay (ELISA) by *Carica* species homogenate through sequential loading of the species leaf homogenates and a standard PRV homogenate

	Loading Sequence	
	1) Species homogenate 2) PRV standard E405	1) PRV Standard 2) Species homogenate E405
C. papaya	0.299	0.634
C. cauliflora	0.172	0.789
C. pubescens	0.076	0.541
C. microcarpa	0.058	0.495

Mean values from 3 replications at 1:30 dilution

E405 for PRV-infected *C. papaya* = 0.862

E405 for healthy *C. papaya* = 0.037

E405 for healthy *C. pubescens* = 0.021

E405 for healthy *C. microcarpa* = 0.026

E405 for healthy *C. cauliflora* = 0.031

available for virus loading when PRV standard was added in the second loading. In wells that were loaded first with papaya or C. cauliflora leaf homogenate the concentration of "IC" was too low to tie up all of the antibody, and a moderate ELISA reaction occurred upon adding PRV in the second loading.

The activity of the "IC" component may not be limited to the γ-globulin. It may also react with PRV particles, but to a lesser extent. This was shown when the PRV standard was loaded into the γ-globulin coated wells before the Carica specie leaf homogenates in the sequential loading experiment (Table 20). The "IC" appeared to block the formation of the antibody-virus "sandwich" by attaching to the PRV. Lower extinction values were observed in wells that had C. microcarpa and C. pubescens in the second loading.

The concentration effect of the "IC" component was demonstrated by mixing a PRV standard (1:50 dilution), with the same volume of a 1:50, 1:100, 1:200 and 1:400 dilution of a homogenate of healthy C. pubescens leaf. The final PRV- C. pubescens mixtures represent 1:100, 1:200, 1:400 and 1:800 C. pubescens leaf-to-buffer dilutions (Table 21). The interference with ELISA was noticeably reduced as the concentration of the C. pubescens leaf homogenate in the mixture was reduced; the greater the dilution factor for C. pubescens, the greater the extinction value observed in ELISA with the PRV-C. pubescens mixture. The damping effect on ELISA by C. pubescens leaf homogenates was observed in dilutions as high as 1 part C. pubescens to 800 part buffer (w/w) (Table 21).

If the "interference component" in the Carica species is protein specific, the addition of ovalbumin in the extraction buffer will

Table 21--Mean extinction values (E405) for the mixing of a PRV standard with different concentrations of a healthy C. pubescens leaf homogenate

Final dilution	PRV infected papaya at 1:50 dilution + C. papaya E405	C. pubescens E405
1 : 100	1.095	0.569
1 : 200	1.045	0.722
1 : 400	1.034	0.881
1 : 800	1.045	0.988

E405 for healthy papaya at 1:50 dilution= 0.148

E405 for healthy C. pubescens at 1:50 dilution= 0.138

E405 for PRV infected papaya at 1:50 dilution=1.459

Mean values from 3 replications

Values measured by "Titertek Multiskan" photometer at 405 nm, 300ul of final substrate volume.

neutralize some of the interference effects. The addition of 0.2% ovalbumin to the potassium phosphate + EDTA buffer in 1:50 leaf:buffer dilutions resulted in positive extinction values for one inoculated C. cauliflora (345) and two uninoculated controls (345, 415). The average extinction value in the inoculated sample was twice as high as in the uninoculated controls (Table 22). Bioassays using this in inoculated C. cauliflora (345) did not induce PRV-infections in Carica papaya. The addition of ovalbumin did not change the ELISA result for the other wild Carica species (Table 22). Since Carica X heilbornii was the only wild Carica species that was susceptible to PRV but had very low extinction values in ELISA, a leaf homogenate of PRV infected C. X heilbornii was prepared in extraction buffer with 0.2% ovalbumin added. Dilutions of 1:50, 1:100 and 1:200 from this homogenate were compared with the same dilutions prepared in buffer without ovalbumin. The ELISA results indicated that neither the addition of ovalbumin nor the dilution of the homogenate increased the extinction values for virus-infected C. X heilbornii. The fact that homogenate dilutions did not help in this case was probably due to a low virus concentration in the C. X heilbornii plant.

The addition of 2% polyvinylpyrrolidone (PVP-40) with or without 0.2% ovalbumin to the extraction buffer did not change the ELISA result for all the wild Carica species.

The present ELISA procedure is not suitable for PRV assay in species other than C. papaya. Before ELISA can be used successfully to diagnose PRV in wild Carica species, a new extraction buffer is needed

to eliminate the interfering substances which are apparently present in leaves of these species.

Table 22--Mean extinction values (E 405) for Enzyme-Linked Immunosorbant Assay (ELISA) of 11 *Carica* species and hybrids for Papaya Ringspot Virus.

	Inoculated E405	Uninoculated E405
C. papaya	1.066	0.120
C. monoica	0.121	0.140
C. stipulata	0.100	0.110
C. quercifolia	0.111	0.128
C. microcarpa	0.099	0.115
C. goudotiana	0.099	0.101
C. X heilbornii	0.188	0.119
C. cauliflora		
345 T1	0.145	-----
345 T2	0.488	-----
345 T3	-----	0.254
415 T1	0.118	-----
415 T2	0.104	-----
415 T3	-----	0.184
C. pubescens	0.106	0.110
C. pubescens x *C. monoica*	0.113	-----
C. goudotiana x *C. microcarpa* T1	0.127	-----
T2	0.124	-----

Mean values from 2 replications

V. SUMMARY AND CONCLUSIONS

Papaya ringspot virus (PRV) was effectively transmitted to Carica papaya by mechanical inoculation with virus infected leaf homogenates. Repeated inoculations during the early part of each planting assured all breeding materials were infected with the virus. Virus tolerance in the breeding populations was rated according to the severity of disease symptoms. Six symptom categories were classified in this study and each of them was rated visually on a 4 point scale.

'Waimanalo' is the most susceptible Hawaiian solo cultivar. Infected trees developed severe fruit, leaf and stem symptoms, and tree growth ceased completely. When 6-week-old 'Waimanalo' seedlings were inoculated with PRV, water-soaked lesions were observed on stems within ten days. This rapid development of severe infection symptoms made 'Waimanalo' papaya a suitable host for PRV bioassay. 'Kapoho Solo' plants also produced severe disease symptoms when infected with PRV, but tree growth was not completely inhibited. Infected 'Kapoho' trees were moderately vigorous and continued to produce small, distorted fruits. Some of the 'Higgins' plants in the 1983-1984 planting were observed to have a moderate degree of tolerance to PRV disease. Infected trees had much smoother leaf surfaces than infected 'Waimanalo' or 'Kapoho' plants, and they also had fewer lesions on petioles and stems. Fruits produced on infected 'Higgins' were small and often deformed.

No papaya plants screened in this project were immune to PRV infection. Line 356, a dioecious line provided by Dr. Robert Conover, was the only selection with a useful degree of virus tolerance. Upon

infection by the virus, selected 356 plants produced light chlorosis along the leaf veins, with mild stem lesions and fruit ringspots. No fruit distortion was ever observed. Line 356 plants were, however, highly susceptible to root rot diseases.

The virus tolerance in the 356 female plants was readily passed on to the 356 x solo papaya hybrids. With the exception of the fruit distortion symptom, virus tolerance in the F1 was intermediate between the tolerant and susceptible parents, and tolerance appeared to be quantitatively inherited. Distortion of fruits was not observed in Line 356 and rarely occurred in the hybrid lines that had 356 as the female parent. The fruit distortion symptom in the solo papayas appeared to be a recessive character, and was suppressed when crossed with 356.

Line 402DF1 was produced by crossing 356R12T8 and 'Kapoho Solo'. Selected trees of 402DF1 and 402DF2s were vigorous, productive and had good virus tolerance. They produced fruits with good color, adequate total soluble solids (13-15%) and usually with a mild acid taste. This acidity gave the fruit a distinctive flavor, which was preferred by some, but disliked by others in a general opinion poll.

Line 410AF1 was produced by a cross between 356R7T7 and 'Higgins'. Selected 410AF1 and 410AF2 plants were compact, and had high tolerance to PRV, although fruit quality was poor (mean T.S.S.=10.5%).

Line 403F1 was produced by a cross between 356R3T3 and 'Waimanalo'. Not many trees were selected from this cross since in general, they were not very vigorous and displayed more severe infection symptoms. Fruit distortions were more common in line 403 than the other 356 x solo

papaya hybrids. However, the occurrence of fruit distortion was still much lower than that in 'Waimanalo' or 'Kapoho solo'.

A total of 17 different F2 trees from 356 x solo papaya hybrids was selected in this study. Selected trees from line 410AF2 had the highest virus tolerance, but fruit quality and yield were poor. A few selected 402DF2 plants were very vigorous and productive; fruits from these plants also had good eating qualities.

A recurrent selection breeding program, using the selected F1 and F2 trees should be pursued for improvement in both fruit quality and tolerance to PRV. The backcross breeding method will not be suitable for this purpose. Backcrossing to the solo parents will dilute the virus tolerance in the next generation, and backcrossing to the virus tolerant 356 plants will further decrease the horticultural qualities.

Interspecific hybridization between C. papaya and other Carica species produced no viable seeds. The cool climate at the Olinda station on Maui provided good growing conditions for the wild Carica species, but it did not lead to success in interspecific crosses with C. papaya. Seeds produced from crosses between C. papaya and wild Carica species were small and appeared to have aborted during the early stages of development. However, fruits remained on the trees until they had ripened. The cool temperatures also caused a delay in ripening in solo papaya fruits. Some hermaphrodite fruit remained on trees for as long as 9 to 12 months after pollination.

Viable plants were produced from reciprocal crosses between C. pubescens and C. monoica and between C. microcarpa and C. monoica. Plants were also produced from crosses between C. goudotiana and C.

microcarpa, and between C. goudotiana and C. pubescens. The authenticity of these hybrids will need to be confirmed through isozyme electrophoresis.

Enzyme-linked immunosorbant assay (ELISA) successfully detected the presence of PRV in infected Carica papaya, but an improvement in the virus extraction buffer procedure will be needed before this technique can be used for PRV assays in other Carica species. The virus produced severe disease symptoms on leaves and stems of inoculated C. X heilbornii. When 'Waimanalo' seedlings were inoculated with C. X heilbornii leaf homogenate, 3 out of 6 plants produced infection symptoms. However, the ELISA extinction value (E405) for the infected C. X heilbornii was only slightly higher than that for an uninoculated control. Non-specific color development was observed in leaf homogenates from healthy C. cauliflora (345,415) when 0.2% ovalbumin was added to the extraction buffer.

Leaf homogenates of C. pubescens and C. microcarpa contained a high concentration of a certain component that inhibited detection of PRV by ELISA. This component is sensitive to high temperature; boiling the leaf samples from C. pubescens and C. microcarpa significantly reduced its effect. However, boiling leaf samples from the Carica species will also denature the virus particles that might be present.

The interference effect on ELISA caused by C. pubescens was observed in dilution as high as 1 part of C. pubescens leaf to 800 parts of buffer (w/w).

Addition of 2% polyvinyl-pyrrolidon (PVP) and/or 0.2% ovalbumin in the extracting buffer did not change the ELISA results for the wild

Carica species. Addition of ovalbumin to 1:50, 1:100 and 1:200 dilutions of PRV infected *C.* X *heilbornii* did not increase the extinction value for this plant.

More research will be needed to identify the nature of the interfering component in *Carica* species.

VI. LITERATURE CITED

Adsuar, J. 1946. Studies on virus diseases of papaya in Puerto Rico. 1. Transmission of Papaya Mosaic. Univ. Puerto Rico Agr. Exp. Sta. Tech. paper 1.

Adsuar, J. 1972. A new virus disease of papaya, Carica papaya, in Puerto Rico. J. Agr. Univ. Puerto Rico. Vol. LVI #4: 397-402.

Awada, M., and Suehisa, R. 1970. Nutrient removal by papaya fruits. Hortsci. 5(3): 182.

Broadbent, L. 1949. Factors affecting the activity of alatae of the aphids Myzus persicae Sulzer and Brevicoryne brassicae L. Ann. Appl. Biology 36: 40-62.

Broadbent, L., and Martini, C. 1959. The spread of plant viruses. Adv. Virus Res. 6: 93-135.

Capoor, S. P., and Varma, P.M. 1948. A mosaic disease of Carica papaya L. in the Bombay Province. Current Sci. 17: 265-266.

Chen, M.J., and Wey, G. C. 1979. Identification, purification and electron microscopy of papaya ringspot virus. In symposium on virus diseases of fruits and vegetables. Homestead, Florida, August 16-18, 1979.

Clark, M.F., and Adams, A.M. 1977. Characteristics of the microplate method of enzymes-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.a

Cook, A. A., and Milbrath, G.M. 1971. Virus disease of papaya on Oahu, Hawaii and identification of additional diagnostic host plants. Plant Disease Reporter 55(9): 785-788.

Cook, A. A., and Zettler, F. W. 1979. Susceptibility of papaya cultivars to papaya ringspot and papaya mosaic virus. Plant Disease Reporter 54: 893-895.

Conover, R. A. 1962. Virus diseases of the papaya in Florida (Abst.). Phytopathology 52: 6.

Conover, R. A. 1964. Distortion ringspot, a severe virus disease of papaya in Florida. Proc. Fla. Soc. Hort. Sci. 77: 440-444.

Conover, R. A. 1976a. A program for development of papaya tolerant to the distortion ringspot virus. Proc. Fla. Stat. Hort. Soc. 89: 229-231.

Conover, R. A. 1976b. Evaluation of methods for detection of tolerance to distortion ringspot virus in papaya. Ann. Cong. Amer. Soc. Hort. Sic. Tropical Region, Puerto Rico. 25: 51-57.

Conover, R. A., and Litz, R.E. 1978. Progress in breeding papaya with tolerance to papaya ringspot virus. Proc. Fla. Stat. Hort. Soc. 91: 182-184.

Conover, R. A., and Litz, R.E. 1981. Tolerance to papaya ringspot virus in papaya, Carica papaya L. Phytopathology 71 (8): 868 (Abst.).

Costa, A. S., and Muller, G. W. 1980. Tristeza control by cross protection: A U.S.-Brazil cooperative success. Plant Dis. 64: 538-541.

deBokx, J. A. 1965. Host and electron microscopy of two papaya viruses. Plant Disease Reporter. 49: 9.

De Zerpa, D. M. 1967. Innoculation of Carica papaya with distortion ringspot virus under different conditions. Agron. Trop. 17 (4): 361-370.

Fletcher, J.T., and Rowe, J.M. 1975. Observations and experiments on the use of an avirulent mutant strain of tobacco mosaic virus as a means of controlling tomato mosaic. Ann. Appl. Biol. 81: 171-179.

Grogan, R. G., Welch, J. E., and Bardin, R. 1952. Common lettuce mosaic and its control by the use of mosaic free seed. Phytopathology 42: 573-578.

Govier, D. A., and Kassanis, B. 1974. Evidence that a component other than the virus particle is needed for aphid transmission of potato virus Y. Virology 57: 285-286.

Govier, D. A., Kassanis, B., and Pirone, T. P. 1977. Partial purification and characterization of the potato virus Y helper component. Virology 78: 306-314.

Gonsalves, D., and Ishii, M. 1980. Purification and serology of papaya virus. Phytopathology 70 (11): 1028-1032.

Harison, B. D., Finch, J.J., Gibbs, A.J., Hollings, M., Shepherd, R.J., Valenta V., and Wetter, C. 1971. Sixteen groups of plant viruses. Virology 45: 356-363.

Higa, S., and Namba, R. 1971. Vectors of the papaya mosaic virus in Hawaii. H. A. E. S. Journal Series # 1253: 93-96.

Higgins, J. E., and Holt, V.S. 1914. The papaya in Hawaii. H. A. E. S. Bulletin 32, 44p.

Hollings, M. and Brunt, A.A. 1981. Potyviruses, In Handbook Of Plant Virus Infections And Comparative Diagnosis. E. Kurstake (ed.) Elsevier/North Holland Biomedical Press. 1981.

Holmes, F. O., Hendrix, J.W., Ikeda, W., Jensen, D.D., Lidner, R.C.,and Story, W.B. 1948. Ringspot of papaya (Carica papaya L.) in Hawaiian Islands. Phytopathology 38(4): 310-312.

Holtzmann, O. V. and Hine, R.B. 1965. Diseases Of papaya Carica papaya in Hawaii. H.A.E.S. Bulletin # 136.

Holtzmann, O. V. and Ishii, M. 1963. Papaya mosaic virus reduces quality of papaya fruits. Hawaiian Farm Sci. 12 (4): 142.

Horovitz, S. Y., Jimenez, H. 1967. Cruzamiento intezespecificos e intergenericos en Caricaceae y sus inplicaciones fitotecnicas separata de la revista. Agronomia Tropical 4 (3): 229-249.

Ishii, M. 1972. Observations on the spread of papaya ringspot virus in Hawaii. Plant Disease Reporter Vol. 56 # 4.

Ishii, M. and Holtzmann, O. 1963. Papaya mosaic diseases in Hawaii. Plant Disease Reporter 47 (11): 947-951.

Ishii, M., Yee, W., and Holtzmann, O. Beware papaya mosaic disease. C. E. S. University of Hawaii. Leaflet 95.

Jensen, D. D. 1949a. Papaya virus diseases with special reference to papaya ringspot. Phytopathology 39: 191-210.

Jensen, D. D. 1949b. Papaya ringspot virus and its insect vector relationships. Phytopathology 39: 212-220.

Jimenez, H. and Horovitz, S. 1958. Cruzabilidad entre especies de Carica. Agron. Trop. 7(4): 207-215.

Khurana, S.M.P. 1970. Effect of virus diseases on latex and sugar contents of papaya fruits. J. Hort. Sci. 45(3): 295-297.

Khuspe, S., Hendre, R., Mascarehas, A., Jagannathan, V., Thombre, M., and Joshi, A. 1980. Ultilization of tissue culture to isolate interspecific hybrids in Carica L. In national symposium of plant tissue culture, genetic manipulation and somatic hybridization of plant cells. Bhabha atomic research center, Bombay, India.

Kuo, M.S., Wang, H.L. and Wang, C.C. 1979. Some observations on spread, vector density, and location of papaya plantation in relation to the occurrence of papaya ringspot in Taiwan. In symposium on virus diseases of fruits and vegetables. Homestead, Florida, August 16-18, 1979.

Lindner, R.C., Ikeda, W., and Awada, M. 1945. Papaya ringspot. H. A. E. S. Biennial Report 1944-1946, 118-119.

Mekako, H.U., and Nakasone, H.Y. 1975. Interspecific hybridization among 6 Carica species. J. Am. Soc. Hort. Sci. 100 (3): 237-242.

Nakasone, H. Y. 1979. The papaya virus situation in Hawaii. In symposium on virus diseases of fruits and vegetables. Homestead, Florida, August 16-18, 1979.

Namba, R. and Higa, S.Y. 1972. Susceptibility of varieties of papaya and cucumber to the papaya mosaic virus when transmitted by the green peach aphid. Proc. Haw. Ento. Soc. Vol. 21 (2): 235-238.

Namba, R. and Higa, S. Y. 1975. Papaya mosaic virus transmission as affected by the duration of preliminary fasting and virus acquisition feeding of Myzus persicae. H. A. E. S. Journal Series 1660.

Namba R. and Higa, S.Y. 1977. Retention of the inoculativity of the papaya mosaic virus by the green peach aphid. Proc. Haw. Ento. Soc. 22(3): 491-494.

Namba, R. and Kawanishi, 1966. Transmission of papaya mosaic virus by the green peach aphid. J. Econ. Ento. Vol 59 (3): 669-671.

Owens, P. C. 1957. The effect of infection with Tobacco Etch Virus on the rates of respiration and photosynthesis of tobacco leaves. Ann. App. Biol. 45: 327-331.

Parris, G. K. 1938. A new disease of papaya in Hawaii. Proc. Amer. Soc. Hort Sci. 36: 263-265.

Pirone, T. P. and Megabed, E. 1966. Aphid transmissibility of some purified viruses and viral RNA. Virology 30: 631-637.

Sawant, A. C. 1957. Crossing relationship in the Genus Carica. Evolution 12: 263-266.

Sawant, A. C. 1958. A study of the interspecific hybrid, C. monoica x C. cauliflora. Amer. Soc. Hort. Sci. 71: 330-333.

Su, H. J. and Lin, C. C. 1979. Preliminary study on the strains of Papaya Ringspot Virus and their cross protection. For symposium on virus disease of fruits and vegetables. Homestead Florida, August 16-18, 1979.

Tayler, C. E. and Robertson, W. M. 1974. Electron microscopy evidence for the association of Tobacco Severe Etch Virus with the maxillae in Myzus persicae (Sulz). Phytopath. Z. 80: 257-266.

Trujillo, E. E. and Gonsalves, D. 1967. Tomato Spotted Wilt Virus in papaya (Abst.). Phytopathology 57: 9.

van der Plank, J. E. 1944. Production of seed potatoes in a hot, dry climate. Nature 153: 589-590.

Warmke, H. E., Cabanillas, E., and Cruzado, H. J. 1954. A new interspecific hybrid in the Genus Carica. Proc. Soc. Hort. Sci. 64: 284-288.

Warner, R. M. 1972. A catalog of plants in the plant science instructional arboretum. University of Hawaii, Misc. Pub. 98. October 1972-5M.

Watson, M. A. 1938. Further studies on the relationship between Hyoscyamus Virus 3 and the aphis Myzus persicae Sulz. with the special reference to the effects of fasting. Proc. R. Soc. Lond. B. 125: 144-170.

_____ 1946. The transmission of Beet Mosaic and Beet Yellows Viruses by aphids; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proc. R. Soc. Lond. B. 125: 144-170.

Wolf, H. S. and Lynch, S. L. 1940. Papaya culture in Florida. Flora. Ag. Exp. Sta. Bul. 350. 35pp.

Wolfenbarger, D. D. 1966. Incidence distance and incidence-time relationship of papaya virus infection. Plant Disease Reporter 50: 908-909.

Yeh, S.D. and Gonsalves, D. 1984. Evaluation of induced mutants of Papaya Ringspot Virus for control by cross protection. Phytopathology V.74(9): 1086-1091.

Zettler, F. W., Edwardson, J. R., and Percifill, D. E., 1968. Ultra microscopic difference in inclusions of Papaya Mosaic Virus and Papaya Ringspot Virus correlated with differential aphid transmission. Phytopathology 58: 332-335.